"Two Component Preparation of Fibrin Glue and Its Clinical Evaluation in Split Skin Grafting"

- A Prospective Study

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Department of General Surgery

Coimbatore Medical College Hospital

Coimbatore - 641018

CERTIFICATE

This is to certify that this dissertation titled ***" Two Component Preparation Of Fibrin Glue And Its Clinical Evaluation In Split Skin Grafting – A Prospective Study"*** submitted to the Tamil Nadu Dr. M. G. R Medical University, Chennai in partial fulfillment of the requirement for the award of M. S Degree Branch – I (General Surgery) is a bonafide work done by **Dr. Pavan A P**, post graduate student in general surgery under my direct supervision and guidance during the period of November 2012 to November 2013.

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DECLARATION

I hereby declare that the dissertation entitled ***"Two Component Preparation Of Fibrin Glue And Its Clinical Evaluation In Split Skin Grafting – A Prospective Study "*** was done by me at Coimbatore Medical College & Hospital, Coimbatore – 641018, during the period of my post graduate study for M.S. Degree Branch-1 (General Surgery) from 2011 to 2014.

This dissertation is submitted to the Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the University regulations for the award of M.S. Degree in General Surgery.

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“Two Component Preparation of Fibrin Glue and Its Clinical Evaluation in Split Skin Grafting”

– A Prospective Study

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Introduction

“Two component preparation of fibrin glue and its clinical evaluation in split skin grafting”

Abstract:

Management of a disrupted tissue is the core of the research in this era and conventional suturing and stapling is painful, time consuming and expensive, tissue adhesives has erupted as a light to this dark. Fibrin glue used in this study is a biological tissue adhesive and is chosen to check for its anchorage, graft uptake, soakage and infection rate. Its two components are fibrinogen and thrombin. Fibrinogen is obtained from cryoprecipitate and thrombin from screened donor’s fresh frozen plasma and the same is applied to 30 patients in the study undergoing split skin grafting. The results were compared to conventional suturing and staples. The fibrin glue tested in the study is a simple, efficacious, cost-effective, less time consuming, better graft uptake, less seroma formation, minimum infection, easy and comfortable intra-operative and post-operative care and patients can be made ambulant early.

Key words: Sutures, staples, fibrin glue, fibrinogen, thrombin, cryoprecipitate, fresh frozen plasma, graft uptake, seroma, infection.

INTRODUCTION

"We expose it, cover it, paint it, tattoo it, scar it, and pierce it. Our intimate connection with the world, skin protects us while advertising our health, our identity and our individuality".

Skin is the outer covering of vertebrates and the largest organ of our integumentary system which is ectodermal in origin and is made up of multiple layers and thereby covers and guards the underlying structures such as muscles, ligaments, bones and internal organs. The most important of all is that, skin is an indispensible barrier which protects us all through the life, from birth to death against physical, chemical and mechanical stress.

Though skin ages as life fades off, but it proves no threat to a person except for the insult on the psychology of a person and this change is inevitable. With the changing population and lifestyle and the intrusion of many diseases in developing countries like India, besides the skin being the most superficial part and having the multi-tasking capacity, it is exposed to a lot of infections, diseases and injuries.

With the exploding population and road traffic accidents, traumatic injury to the skin has also become very much common. With raising epidemic and India becoming the diabetic capital of the world, diabetic ulcer has been a common and challenging problem to avoid and to treat.

Skin grafting came as a major breakthrough in treating ulcers and raw areas which cannot be closed primarily and is still evolving. Way back in 1804, **Baronio** performed the first skin grafting on a lamb. This led to the evolution of skin grafting where the first auto transplantation of the skin was done by placing a 2-3mm epidermal graft on a granulating wound, which was done by **Jacques Reverdin**, a Swedish medical student in Paris in 1869.

His experiment not only demonstrated that the skin transferred from one part of an individual to other part of the same individual can survive but also showed that it can hasten wound healing.

Goerge Lawson was the first person to perform a full thickness graft successfully in 1871 followed by a French surgeon, **Louis Ollier**, who performed intermediate thickness graft as large as 8 cm^2 in 1872.

Carl Thiersch, a German surgeon in 1874 was first one to recognize the importance of preparing the recipient bed by removing the granulation tissue over

the wound before grafting, which dramatically improved the graft take and he erroneously thought taking thin grafts was required for better and faster healing of the donor site.

Even after experimenting on skin grafts over a century, many surgeons were not happy to go about the procedure because of the unreliability, difficulties faced while harvesting the grafts and some even suggested that it results in two wounds instead of one, until **Otto Lanz** described the method of meshing the grafts that increased the length of the graft which can be used for the recipient and the donor area.

In 1929, **Vilray Papin Blair** and **James Barrett Brown**, from Saint Louis differentiated split thickness, intermediate and full thickness grafts with reliable results with the advantages and disadvantages of each. They proposed a theory which holds true till today about the wound preparation, graft harvest, graft application, contraindications and post operative care.

This was followed by the Era of development of instruments for harvesting the grafts. In 1936, the first device was specifically built for harvesting a graft, the **Humby's** knife that contained a razor with a guard which prevented the surgeon from cutting the harvest deeper. For the faster and precise harvests, dermatome

was developed, but the first power driven dermatome developed by **James Barrett Brown**, in 1945 is the most popular till date.

Once the graft is harvested, it can be fixed to the recipient bed using good old conventional method of sutures and staples. Due to the difficulties, disadvantages and skills mandated by the conventional method of suturing and stapling the graft, the idea of developing the tissue adhesive was then floated.

In 1909, BERGEL first reported use of fibrin as the biological adhesive, but TIDRICK and WARNER in 1944 used fibrin glue for fixing skin grafts.

Fibrin glue is an alternative to conventional suturing and stapling of skin grafts and has some added advantages. Conventional methods had some demerits like foreign body reaction, scarring, infection, pain during removal and was expensive too, whereas the use of fibrin glue is simple, safe and cost-effective method, with a rapid technique to fix the skin graft, avoid per-operative bleeding and post-operative collection, better uptake of graft and better overall results.

This biological Fibrin glue initiates the final coagulation cascade, when human fibrinogen is activated by thrombin. Fibrin glue is a sealant and a hemostatic agent with an added adhesive property that has been reportedly used in many specialties with better outcomes.

Many techniques have been developed to produce fibrin which is commercially available and these have their own risks, expensive, cumbersome and time consuming.

In this study, fibrin glue which we have advocated includes Cryoprecipitate, which is a rich source of fibrinogen and thrombin from screened healthy donor's fresh frozen plasma, is less time consuming, cost-effective and safe and the same has been used in thirty patients admitted in Coimbatore medical college and hospital, Coimbatore. This study compares the effectiveness of fibrin glue with conventional sutures and staples.

Aim of the study

AIM OF THE STUDY

OBJECTIVES:

"To study the effectiveness of fibrin glue in anchoring the split skin graft to the wound bed and to analyze the advantages, disadvantages and outcome of using fibrin glue compared to suturing and stapling".

Review of literature

REVIEW OF LITERATURE

It is the largest human organ with a complex and fascinating structure and physiology and it is continuous with the epithelium of the respiratory, digestive and uro-genital tract.

The skin protects us by acting as a barrier against a vast number of destructive forces like chemical absorption (as epidermis acts as a semi permeable membrane), fluid loss, solar radiation and infectious agents and its dermal durability resists physical forces as well.

It plays an important role in sensation and internal metabolism. It acts as body's primary thermoregulatory organ by regulating the body's heat, which makes skin as a fascinating and one of the best studied tissues of the human body for analysis.

SKIN ANATOMY AND PHYSIOLOGY:

Skin is the largest organ of the body. It is continuous with the epithelium of the respiratory, digestive and uro-genital tract. It plays an important role in the sensation and vitamin D metabolism.

Skin consists of two layers, an avascular epidermis and a vascular dermis. The main function of epidermis is to build a tough layer of dead cells by a process called as Cornification, so that it can withstand the insult of the environment. Dermis is a vascular bed to the epidermis and has capillaries which regulate the temperature.

Epidermis:

It is the superficial layer and its thickness varies from 0.04 mm on the eyelid to 1.6mm over the palm. It contains distinct cells such as keratinocytes, melanocytes, Langerhans cells and Merkel cells.

Embryology:

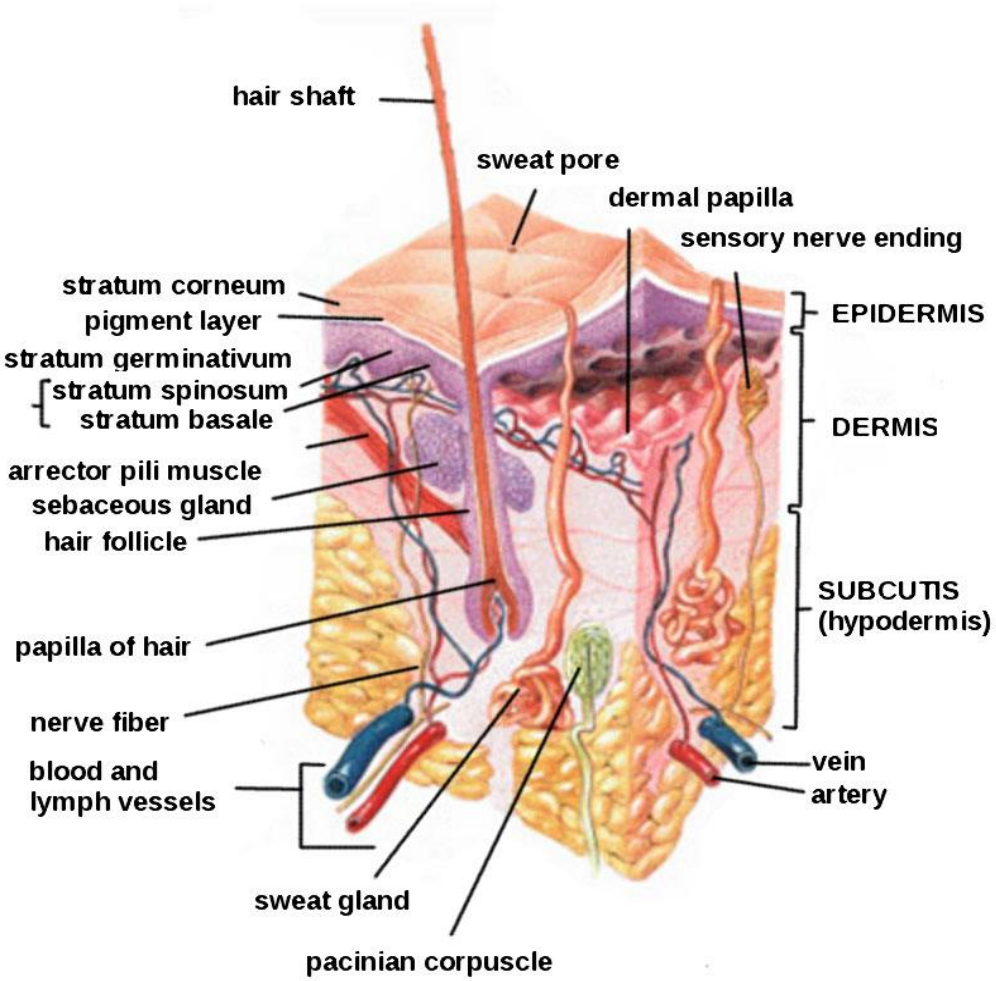
It first appears at the 3rd week of gestation, as a single layer and it is a derivative of ectoderm. It then splits into basal germinal layer and outer glycogen rich flatter layer called Periderm, by 4th week of gestation. The skin is cornified completely by 6th month of gestation and a five layered distinct epidermis comes into existence.

The five layers are: Stratum Germinativum or basal layer, Stratum Spinosum or prickle cell layer, Stratum Granulosum, Stratum Lucidum and Stratum Corneum. Only the palms and soles have all the five layers and the rest of

the body contains only two layers, the stratum corneum and stratum germinativum respectively.

The melanocytes appear by 8th week of gestation and are derived from the neural crest cells. Melanocytes transfer melanosomes (melanin) to the keratinocytes by the 5th month of gestation.

The Langerhans cells are derived from haematopoetic cells and Merkel cells are thought to be derived from neural crest or from the ectoderm.



Functions:

The primary function of the skin is to act as a barrier and give the protection against the environment and organisms. A layer of dead cells covers the organism by the cornification process and acts as a barrier. Basal layer contains columnar or cuboidal cells, where the cornification begins. And these cells have large oval nuclei and basophilic cytoplasm.

Tono filaments are synthesized in the basal cells. Then they get aggregated and ascend through the epidermis and form a keratinous protein which gets accumulated in the cell to form a mature keratinocyte or corneocyte.

In stratum spinosum, the cells are polygonal in shape and resemble spines in light microscopy due to the cell to cell connections called Desmosomes.

Keratinohyalin granules are produced by keratinocytes in the stratum granulosum and these granules contain histidine rich cationic protein called Profilaggrin, which gets degraded to Filaggrin. This Filaggrin acts like a glue that holds the keratinofilaments together. Second type of granules called lamellar granules contains free sterols, polar lipids and several hydrolytic enzymes. These granules are thought to give the skin its impermeable quality, by establishing a hydrophilic crystalline sheet within the intercellular space in the cornified layer.

Marginal band or the cornified cellular envelope is a thickening that develops along the top of the keratinocytic plasma membrane, which is formed by the disulphide and the other chemical bonds between keratolinin and involucrin. The multiple neutral lipids provide a strong integrity to the epidermis to withstand the chemical and physical insults of the environment.

Basal layer of the epidermis consists of melanocytes in the ratio of one melanocyte to every ten keratinocytes. The main function of the melanocytes is to produce melanosomes, which are vesicles full of melanin.

Melanin is of two types: Pheomelanin, which has characterstic lighter colour ,like blond or reddish and Eumelanin ,which accounts for black or brown colour. The prime function of melanin is to protect skin from the harmful effects of UV rays. Studies also support that melanin acts as a biochemical neutralizer of oxygen free radicals.

The middle layer of epidermis contains specialized cells called Langerhans cells. These Langerhans cells play an important role in immune mechanism by playing a large part in contact dermatitis, allograft rejection and neoplasia surveillance.

Epidermis also consists of specialized cells called Merkel cells which are neurosensory cells. These Merkel cells are found in the epidermis of palms and soles, nail bed, oral and genital epithelium. Merkel cells are found close to the neurites and they act as melanoreceptors and transmit mechanical forces, to generate action potential along the nerve fibers.

Dermis:

It is mainly composed of collagen, elastin fibres and ground matrix. When compared to epidermis, it is relatively non-cellular and consists of nerve, vessels, lymphocytes and glandular elements of the skin. Dermis is 15-40 times thicker than the epidermis but because of its lesser cellularity, it consumes lesser energy than epidermis.

The free cells found in the dermis are fibrocytes, mast cells, histiocytes, Langerhans cells, lymphocytes and rarely eosinophils. Dermis contains two layers namely, a superficial papillary dermis and a deep reticular layer.

The papillary dermis contains disorganized collagen bundles, elastic fibres, fibrocytes, ground substances and richly developed microcirculation. Epidermis does not have inherent blood supply and it gets blood supply from the microcirculation of dermis, for its metabolic activity.

Reticular dermis consists of thick bundles of collagen fibres which are arranged in orthogonal fashion, interspersed by coarse elastic fibres.

Periadenexal dermis: it is the dermis which is present immediately adjacent to the hair follicles, apocrine glands and eccrine glands. It resembles papillary dermis.

Ground substance is primarily composed of mucopolysaccharides, hyaluronic acid and chondroitin sulfate and these are mainly found in the papillary dermis and to a lesser extent in the reticular dermis. This provides the gel like consistency and with aging it is replaced by fibrous tissue.

Dermal – epidermal junction:

It is the specialised site of attachment between the epidermis and the papillary dermis. It mainly contains basal keratinocytes, melanocytes and Merkel cells.

It has four layers when seen under the electron microscopy. First layer is made of hemidesmosomes, second layer- lamina lucida , third layer -lamina densa predominantly made up of type IV collagen and lastly a fibrous zone composed of anchoring fibrils, type III collagen and dermal microfibril bundles.

Extra cellular matrix: Extra cellular matrix fulfils many biological functions such as tissue organization, growth factor reservoir, semi permeable selective barrier

and supporting cell monolayer during the tissue development. It contains anionic proteoglycan such as heparin sulfate which acts as a chemical barrier.

Dermis has dense extra cellular matrix(ECM) that supports the complex network of nerves, vasculature and adenexal structures. Apart from the architectural framework that imparts mechanical support and visco-elasticity , they regulate neighbouring cells along with their ability to migrate, proliferate and survive injury.

Blood supply:

Skin gets its blood supply from the subdermal plexus. Subdermal plexus supplies the skin appendages and ends in the plexus present in the superficial layers of papillary dermis, from where capillary loops arise and supply the epidermis. Blood supply to the subdermal arterial plexus comes from two types of cutaneous perforators, the musculocutaneous perforators arising from the muscle and the direct cutaneous arteries from the segmental perforators.

Venous drainage follows the arterial network.

Graft is devoid of its blood supply, sensory innervations and drainage. So the graft entirely depends upon the recipient bed for survival.

SKIN GRAFT:

Definition:

Grafting is the transfer of tissue from one area to another without its blood supply or nerve supply.

Autograft:

Autograft is the transfer of tissue from one location to another location in the same patient.

Isograft :

The transfer of the tissue between individuals who are genetically identical e.g. transfer between two identical twins.

Xenograft (Heterograft):

The transfer of the tissue between a donor of one species to a recipient of another species.

Skin grafting is the simplest method of wound closure in the reconstruction ladder when wound closure is not possible primarily or closure under tension.

Full thickness grafts are usually applied over small areas and over the face, ears and hands, whereas split thickness graft is preferred over large areas and the trunk and genitalia.

Indications for skin grafting:

1. Any traumatic wound that cannot be closed primarily

2. Defects after oncologic resection

3. Burn reconstruction

4. Scar contracture release

5. Congenital deficiencies of skin, such as syndactaly and vaginal atresia

6. Hair restoration

7. Vitiligo

8. Nipple areolar reconstruction

CONTRAINDICATIONS:

Absolute:

a.Wounds with avascular beds

b. Infected wounds

c. Wounds due to malignant neoplasia

Relative:

a. Pressure sores

b. Wounds due to irradiation

c. Wounds due to vasculitis

d. Wounds due to arterial insufficiency

e. Wounds in cosmetically sensitive areas

f. Malnutrition

Classifications:

Types

A) Partial thickness graft (Split thickness graft or Thiersch graft)

B) Full thickness graft (Wolf graft)

Partial thickness graft:

Partial thickness graft is the removal of whole of epidermis with a portion of dermis from the donor area.

Depending on the dermis thickness it may be

a) Thin SSG
b) Intermediate SSG
c) Thick SSG

Donor sites:

Split thickness graft can be taken from any part of the body, including the scalp region. Since the donor area becomes hyperpigmented or scarred frequently, it is chosen from the hidden areas of the body like thighs, trunk and buttocks. Facial defects are usually covered by full thickness grafts or local flaps. Rarely split skin graft is used which is chosen from the "BLUSH ZONE". Blush zone includes the

area above the shoulder such as supraclavicular region, neck and scalp. Hair bearing region is usually avoided for the simple reason that if too thicker graft is taken, it may grow hairs once it is taken up well.

Men have thicker skin than the women irrespective of the site, whereas infants and elderly individuals have thinner skin. Skin over the trunk and thighs are thicker whereas eyelids and post auricular region have thinner skin.

Donor site care:

Healing of the donor site after harvesting the split skin graft takes about 7-21 days. Usually fine meshed gauze impregnated with lubricants and antibiotics is applied to the donor area after the split skin graft is taken and it is left on the donor site until it falls off.

This lubricated gauze gives protection as well as pain. Bilaminated membrane like BIOBRANE or water permeable, bio-occlusive plastic adhesives like OPSITE can also be used.

Ideal and optimum treatment of the harvest site is by auto grafting, so whenever possible excess skin should be auto grafted rather than discarding it. Cultured keratinocytes sheets, skin allografts and other skin substitutes have been developed to hasten faster healing of the donor site.

Harvesting:

Harvesting a graft can be done using either freehand or a power driven dermatome.**WECK** blade, **HUMBY**'s knife, **BLAIR** knife and simple scalpel are few examples of freehand dermatomes. Advantage of the freehand is that, it can be done quickly without the help of electric or pneumatic power, but one has to be careful in maintaining the exact thickness and depth of the graft.

Harvesting can be done under local anesthesia or regional nerve block. Once the site is chosen, WECK or BLAIR blade is mounted onto HUMBY's knife. Typically the guard is set at 0.3-0.45mm to get the ideal thickness of split skin graft.

Once the instrument is ready, lubricant is applied to the area from where the graft needs to be harvested and the assistant applies tension. The surgeon then passes the knife parallel to the epidermis like a violin, in back and forth direction. Once harvested it is meshed and applied to the recipient area.

First electrically driven dermatome was developed by **JAMES BARRETT BROWN** in 1940. It uses rapidly vibrating blade which moves like a wood planar.

Most commonly used motorized dermatome, AIR ZIMMER dermatome which is powered by compressed water pumped nitrogen, produces uniform grafts of predetermined depth and width.

Power source should be checked before using the power dermatome, until and unless it is battery driven. Usually the skin is harvested under general anesthesia when power dermatome is used, but can be done under local or regional block. Graft is harvested in the same way as the freehand dermatome with few modifications.

Full thickness graft:

Generally scalpel is enough to harvest a full thickness graft. Usually it is preferred for small areas like face. Template is prepared and placed over the site from where the graft has to be harvested. A graft is harvested with the full thickness of the dermis with little or no subcutaneous fat. Donor site can be covered primarily or by split skin graft.

Advantages:

SPLIT SKIN GRAFT	FULL THICKNESS GRAFT
Technically easier to perform	Less contractures
Wide area can be covered	Color match is good
Donor area heals by itself	Sensation, hair follicles, functions of sebaceous glands are retained
Graft take is better	Cosmetically and functionally better

Disadvantages:

SPLIT THICKNESS GRAFT	FULL THICKNESS GRAFT
More contractures	Can be used only over small areas
More seroma and hematoma formation	Donor area needs to be covered
Infection rate is more	Cannot be used to cover ulcers
Loss of hair growth, blunting of sensation	
Dry and scaly skin due to loss of sebaceous glands	

GRAFT SURVIVAL:

The process by which the graft survives are:

a) Plasmatic imbibitions.

b) Graft revascularization or inosculation.

c) Maturation or neovascularisation.

Plasmatic imbibtion:

Aubscher and **Goldman** were the first physicians, who identified the graft survives by serum nourishment and it was called plasmatic circulation.

Once the graft is placed on the recipient bed it becomes edematous and gains weight. Plasma which is leaked mainly from the venules and partly from the arterioles and capillaries get accumulated between the graft and the recipient. This plasma contains fibrinogen, which anchors the graft to the bed and provides nourishment.

Once the graft is placed on the recipient bed, it evacuates its blood and serum. The graft passively absorbs serum from the recipient bed, through the cut ends of

the vessels and porous dermis. Anaerobic metabolism takes place in the graft and this may stimulate revascularization. Endothelial in growth occurs throughout serum imbibition process and vascular flow occurs as quickly as possible. Thus both serum imbibiton and revascularization occurs together but separately.

Graft revascularization:

Thiersch confirmed the process of inosculation in 1874 in histologic sections of full thickness graft. According to this theory, internal vessels of the recipient and the host bed line up and form an anastomosis which provides inflow and outflow to the graft (vascular connections have been shown to begin as early as 22hrs).

Another study done by **Garre , Hubscher, Goldman , Iungengal** and **Endrlen** demonstrated that the original vascularisation of the graft degenerates and endothelial cells and capillary buds from the host bed restores the blood flow by direct invasion.

Third theory proposed by **Henry**, suggested that the graft's vasculature undergoes degeneration but the cellular basal lamina persists and this acts as conduit for the invasion of new blood vessels from the host bed. This was histologically proven by **Henry**, where he demonstrated patent vascular channels after 48 hrs in the skin grafts, which got endothelialised later by the host capillary bed invasion.

Revascularization in the split skin graft can survive for a longer period without revascularization due to the fewer cellular components that are present in the split skin graft than full thickness graft. More so, the thick dermis acts as a barrier and hampers the process of diffusion during the serum imbibition.

Graft maturation:

Graft continues to mature and contract after it is completely vascularised over 6-12 months. Wound and graft contraction is the most significant event to occur during the maturation of the graft. The area which is not grafted will undergo natural healing by contraction mediated by myofibroblast, which occurs actively. Within the wound many fibroblasts differentiate into myofibroblasts and contract the wound, before the wound is grafted. The graft once planted will also contract depending upon the dimensions of the wound. This process is called Secondary contraction. The contraction in the graft which occurs after harvesting, is because of the inherent elastic property and this is called as Primary contraction. This primary contraction is more in the full thickness graft than split thickness graft. This can be easily overcome by stretching the graft before its application. Secondary contraction is well understood than the primary contraction. In secondary contraction dermis inhibits the myofibroblast differentiation, so full thickness graft goes for lesser secondary contraction than the split thickness graft.

This is the reason whenever possible full thickness graft should be applied over the joint, over the face, in the web space of the hand and for the scar contraction surgery. If the defect is large, better to go with split thickness graft.

Apart from secondary contraction, the full thickness graft has an advantage over the split thickness graft that it has the ability to grow, so full thickness graft is preferred especially in pediatric age group.

Epidermis during maturation phase may become 7-8 times thicker than the original by hyperplasia. This is clinically evident as crusting and scaling. This overgrowth of the epidermis will grow along the uncovered portion to aid the healing of the wound.

Hyperpigmentation in heavily pigmented individuals is poorly understood, especially when the graft is placed over palms and soles. This can be avoided by careful selection of the donor area or by avoiding sun exposure to prevent permanent hyperpigmentation. Also hyperpigmentation can be reduced by dermabrasion and the chemical peeling of the skin.

In the initial 30 days, the nerve fibres undergo degeneration within the graft after transplantation. By around 40 days, the newly formed nerve fibres will start invading the graft both from the periphery as well as from the graft base. Among these fibres most of them travel along the Schwann sheaths, that is left of the

original nerves or along the blood vessels. The fibrils multiply in number by 2-3 months and reach the end organs near to the sweat glands, hair follicles and sensory end organs resulting in the crude reformation of the sensory network.

Grafted skin will be hypersensitive for a period of one year, which will be followed by touch, temperature and tactile discrimination. However neural architecture resembles that of the donor area. This sensation will be better and faster in full thickness graft than the split thickness graft.

The functioning of the sweat glands depends on the neural regeneration, hence sweat gland functioning is superior in the full thickness graft than the split thickness graft. It takes 2-3 months for the sweat glands to form sweat. Till then the grafted area will appear dry and scaly. This can be avoided by applying moisturizing cream.

Hair follicle growth is generally seen after full thickness graft and it maintains the property of the donor area. So for male pattern baldness and eye reconstruction, full thickness graft is preferred.

GRAFT FAILURE

Causes of graft failure:

1. Graft placed on an avascular bed

2. Infection

3. Hematoma

4. Seroma

5. Shearing

6. Malnutrition

7. Poor underlying medical condition

8. Upside-down graft or other technical errors

Graft will fail when serum imbibitions or revascularization process is disturbed during the development period. The most important thing for the graft uptake is the healthy and vascularised bed. Inadequate bed is considered as one of the more common causes of the graft failure. Exposed tendons, cartilage and bone are considered as relative contraindications for the skin grafting, since they do not take up the graft well.

Poorly vascularised tissues such as fat, peritoneum, perichondrium and periosteum support split thickness graft well when the granulation tissue is allowed to grow on them.

Blood, serum and pus when present between the graft and the host bed prevent the graft take. It can lead to complete or partial graft failure. This can be avoided to some extent by making vertical slits in the graft.

Another common cause of graft failure is shearing of the graft which occurs during the early post operative period while changing the dressing or mobilization of the extremity by the patient. To prevent it, dressing should be done carefully and the extremity should be immobilized.

Infection when present prevents the graft uptake**, KRIEZK and ROBSON** proved that if the bed contains greater than 10^5 organism per gram of tissue, the survival of the graft can be hampered. Hence organism such as hemolytic streptococci and pseudomonas are considered as contraindications for the skin grafting. **JAMES BARRETT BROWN** and **VILRAY PAPIN BLAIR** suggested the freedom from systemic infection before the skin grafting to prevent failure.

Malnutrition, steroids, vasculitis, malignancy, chemotherapy and immunosuppression can result in unfavorable systemic or local conditions which

can have an effect on graft uptake. Previous radiation injury and pressure effect on the host or the donor site can also prevent total or partial graft take.

Dermatome and meshers too hot after sterilization, when used during the skin grafting can also affect the graft take.

BLOOD COAGULATION:

Blood coagulation theories have existed since antiquity. Fibrin was explained by the physiologist, **Johannes Müller** as a substance of thrombus. **Rudolf Virchow** described its soluble precursor, Fibrinogen and **Prosper Sylvain Denis** chemically isolated it.

The process of the conversion from fibrinogen to fibrin is the result of an enzymatic process, and the labeled the hypothetical enzyme "thrombin" and its precursor "prothrombin" was suggested by **Alexander Schmidt.** The essentiality of calcium in the coagulation of blood was discovered in1890, by **Arthus. Giulio Bizzozero** identified the platelets in 1865 and elucidated their functions in 1882.

Paul Morawitz, in 1905 consolidated the theory that presence of tissue factors is required for generation of thrombin. At this point it was known that the damaged tissue releases thrombokinase/thromboplastin(factor III) which reactes with

prothrombin along with calcium to form thrombin and this converts fibrinogen to fibrin.

Thrombogenesis is the process where blood gets clotted. It is a process, where blood loss from a damaged vessel is controlled by formation of platelet and fibrin containing clot and subsequently the damaged vessel is repaired.

Thrombogenesis (Coagulation) is highly conserved throughout biology and it includes both a cellular (platelet) and a protein component (coagulation factors) and this hemostatic system in humans is very well understood and extensively researched.

Coagulation begins almost instantly when endothelium lining the blood vessel is injured. When the blood is exposed to tissue factors, it initiates changes in the platelets and fibrinogen, wherein the platelets immediately forms plug at the site of injury. This process is called as Primary hemostasis.

Proteins in the blood plasma, called the **coagulation factors or clotting factors**, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug. This is called Secondary hemostasis

Physiology of coagulation:

Formation of the platelet plug:

Platelets play a major role in sealing of the smaller vessels by formation of the platelet plug.

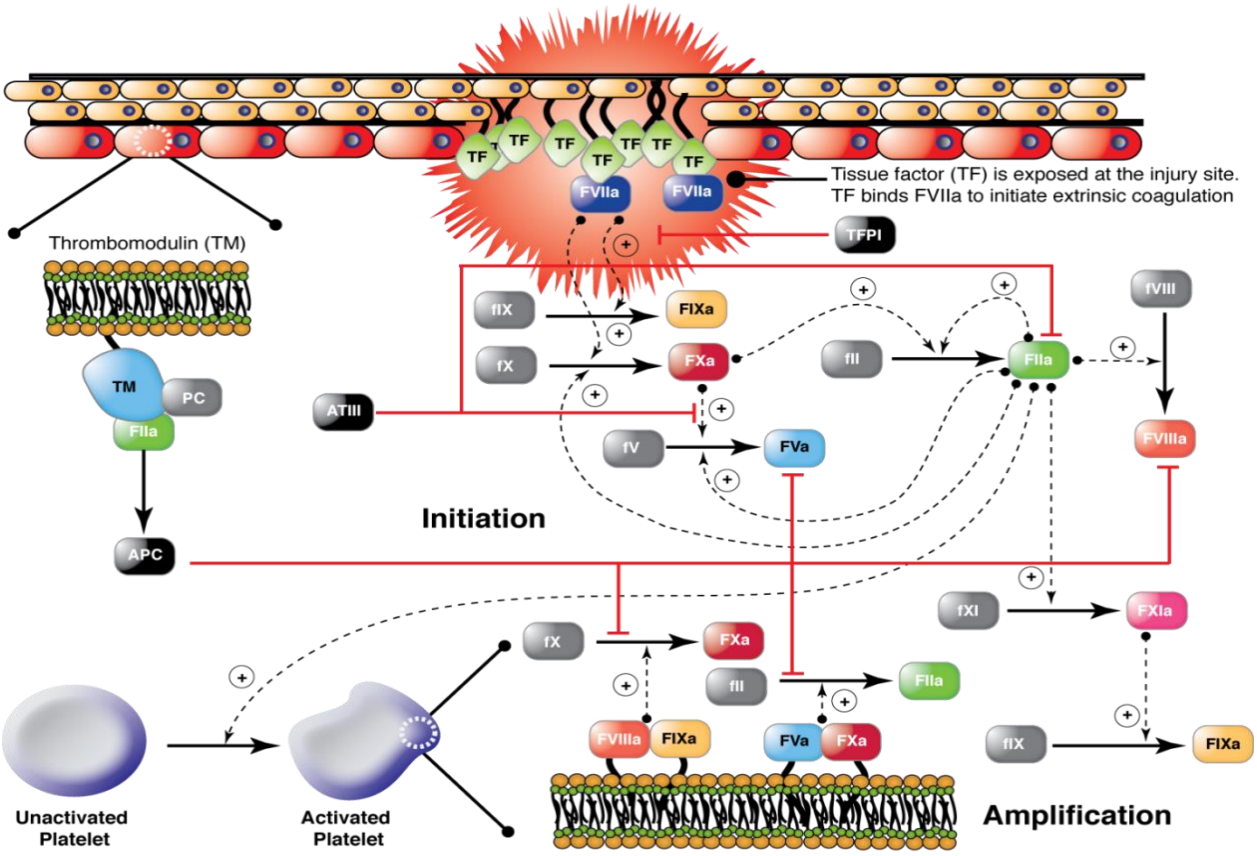
Physical and chemical characteristics of platelets:

- Platelets are derived from megakaryocytes from the bone marrow.
- Size ranges from 1-4micrometer in diameter.
- The normal concentration of platelets in the circulation ranges between 1,50,000-3,00,000/microlitres.
- Half life of platelets in the blood ranges from 8-12 days, which are removed by the tissue macrophages (More than half in spleen).

Functional characteristics:

- Platelets donot have nuclei so they cannot reproduce, still they carry out arrays of functions and their cytoplasm contains:

a) Contractile proteins such as actin and myosin which are similar to those in muscle cells and they also contain thrombosthenin which all together help in platelet contraction.



Blood coagulation in vivo pathway showing central role of thrombin

Note: TF: tissue factor TFPI: tissue factor pathway inhibitor

TXA2: thromboxane A2 TM: thrombomodulin

ADP: adenosine diphosphate ATIII: antothrombin III

PC: protein C APC: activated protein C

b) Golgi apparatus and Endoplasmic reticulum residue store a large quantity of calcium ions and produce many enzymes.

c) Mitochondria and enzyme system of the platelets have the capacity to produce adenosine triphosphate and diphosphate.

d) Prostaglandins are generated by the enzyme systems which acts as local

hormone on the vessels and perform other local reactions.

e) Fibrin stabilizing factor.

f) Contain growth factors which act on vascular endothelial cells, vascular smooth muscle cells and fibroblasts.

The cell membrane of platelets is coated with glycoprotein on their surface which aids in adherence of platelets to the injured endothelial cells and exposed collagen of the vessel wall. It also contains large quantities of phospholipids which initiate blood clotting process.

Mechanism of platelet plug formation:

Once the platelets come in contact with injured vessel, particularly with the collagen, they change characteristics. It swells and attains irregular shape with numerous pseudopods irradiating and protruding from their surface.

The contractile proteins present in the cytoplasm of platelet contract and release granules containing the active factors. The surface of platelets become sticky, which help in adhesion of platelet to the collagen of the vessel wall and von Willebrand factor (protein that leaks from the plasma into the tissue). Platelets release adenosine diphosphate and thromboxane A2, which once released activate other platelets and these additional platelets released become sticky and get adhered to the original activated platelet forming a platelet plug. Initially platelet will be loose and this is enough to seal a small vessel, but in larger vessels blood coagulation occur subsequently forming fibrin threads. These fibrin threads attach tightly to the platelet plug and form an unyielding plug.

Cascade of coagulation:

The Secondary hemostasis of coagulation cascade has two paths which lead to the formation of fibrin and these are the intrinsic pathway (also known as contact activation pathway) and extrinsic pathway (also known as the the tissue factor pathway). Before it was thought that both the pathways has equal importance which joins the common pathway, but now it is known that tissue factor pathway is primarily required for the initiation of blood coagulation.

The coagulation cascade consists of three pathways.

a) The Tissue factor pathway

b) The Contact activation pathway and

c) Both activate the "final common pathway" of factor X, thrombin and fibrin.

Tissue factor pathway or Extrinsic pathway:

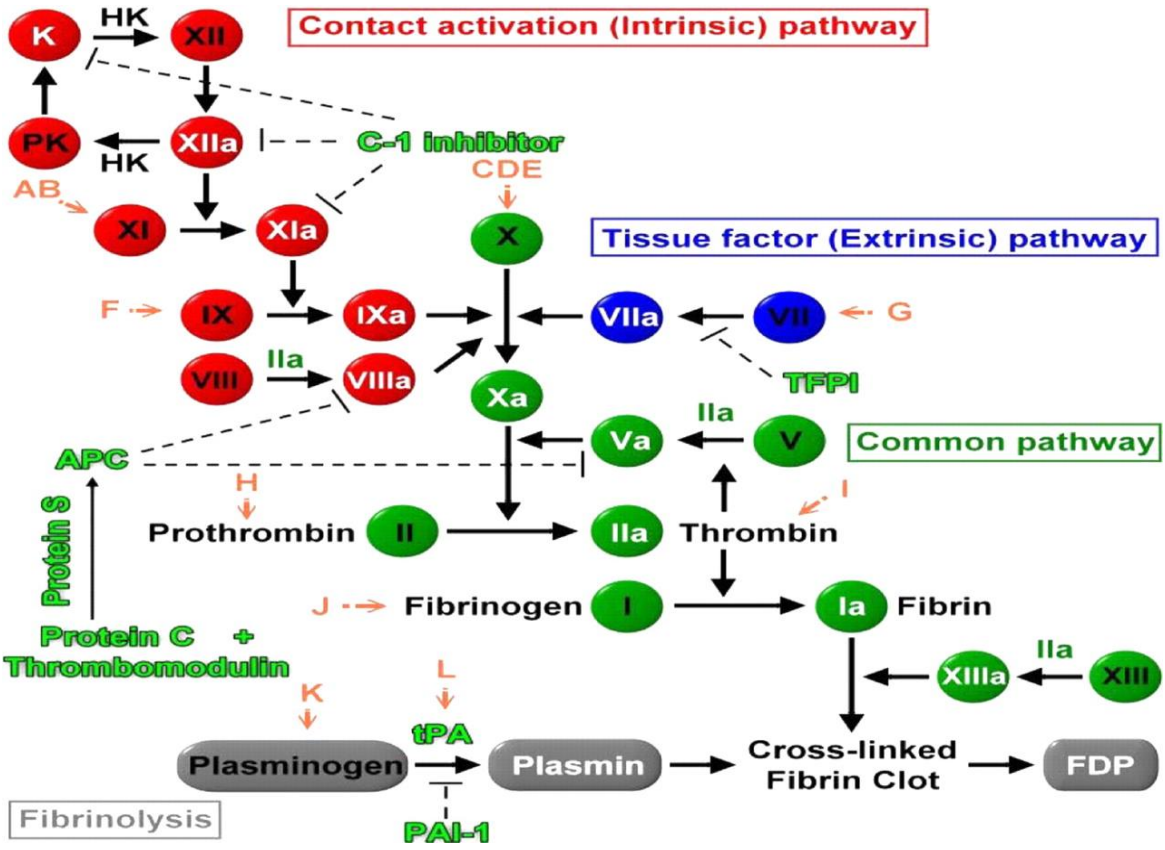
The main aim of this pathway is to generate a "thrombin burst", it is a process wherein thrombin, the crux of the coagulation cascade in terms of its feedbacks, is rapidly released. Among all the activated factors, FVIIa is the one which circulates in highest amount.

It involves

a) Release of tissue factor: tissue factor or tissue thromboplastin released when a vessel is traumatized functions as proteolytic enzymes.
b) Activation of factor X: lipoprotein complex along with FVIIa activate FXa in the presence of calcium ions.
c) Effect of FXa on prothrombin activator and role of FV: Following activation of FX, it immediately combines with tissue phospholipids along with FV to form prothrombin activator. This prothrombin activator in the presence of

calcium ions, splits prothrombin to thrombin and the coagulation process continues.

d) Initially factor V will be in inactive form, the proteolytic activity of thrombin activates factor V and this activated factor V accelerates the prothrombin activation. Thus FVa acts as a protease in the activation of prothrombin activator complex, causing splitting of prothrombin to thrombin. It also accelerates the protease activity of FX and phospholipids which acts as a vehicle in the pathway.



COAGULATION CASCADE

Intrinsic pathway:

a) Activation of factor XII and release of platelet phospholipids: Clotting factors such as FXII and platelets alter their configuration following trauma to the blood or on exposure of collagen. This alteration in molecular configuration of FXII converts itself into a proteolytic enzyme called "Activated factor XII". Damaged platelet release platelet factor 3 which subsequently plays a role in clotting cascade.

b) This activated FXIIa converts FXI to activated FXIa and the conversion requires high molecular weight kininogen (HMWK) and prekallikrein.

c) This activated FXIa in turn activates factor IX.

d) Activation of factor X and role of factor VIII: FIXa acts in concert with FVIII and platelet factor 3 and phospholipid activates factor X. So if factor VIII and platelets are deficient, the coagulation will not be complete.

Final common pathway

Thrombin carries a large array of functions. The main function of thrombin is to convert fibrinogen to fibrin, which is the main foundation of a hemostatic plug. Thrombin in the presence of thrombomodulin also activates Factors VIII and V and their inhibitor protein C. The Factor XIII which is activated forms covalent bonds, that cross link the fibrin polymers that form from the activated monomers.

Until it is down-regulated by the anti-coagulant pathways, prothrombotic state of the coagulation cascade will be maintained by the continued activation of FVIII and FIX to form the tenase complex.

Role of calcium:

Calcium plays a very important role in both intrinsic and extrinsic pathways. Calcium is required throughout the cascade except for the first two steps of intrinsic pathway. This indicates that calcium is required to accelerate the coagulation and in the absence of calcium, either of the pathways will not proceed.

So when blood is drawn for storage in the blood bank and for further transfusion purpose, clotting is prevented by reducing the threshold of calcium ions concentration by deionizing it with citrate ion or it is precipitated with oxalate ion.

FIBRINOLYSIS:

Plasminogen is a plasma protein which when activated gets converted to plasmin. Plasmin is proteolytic enzyme and resembles pancreatic enzyme trypsin. This causes digestion of fibrin fibers and other procoagulants like factor V, factor VIII, factor XII and prothrombin and thereby digesting the clot.

For plasmin to lyse the clot, plasminogen has to be activated. Inactive form of plasminogen will be present in the clot. Once the bleeding is stopped, a few days

later the injured tissue and vascular endothelium will release a powerful factor called tissue plasminogen activator which in turn activates plasminogen resulting in formation of plasmin which ultimately clears off the clot.

Blood clotting tests:

a)Bleeding time:

- Normal: 1 to 6 minutes
- It is prolonged especially in the lack of platelets.
- It is prolonged in the lack of several clotting factors also.

b)Clotting time:

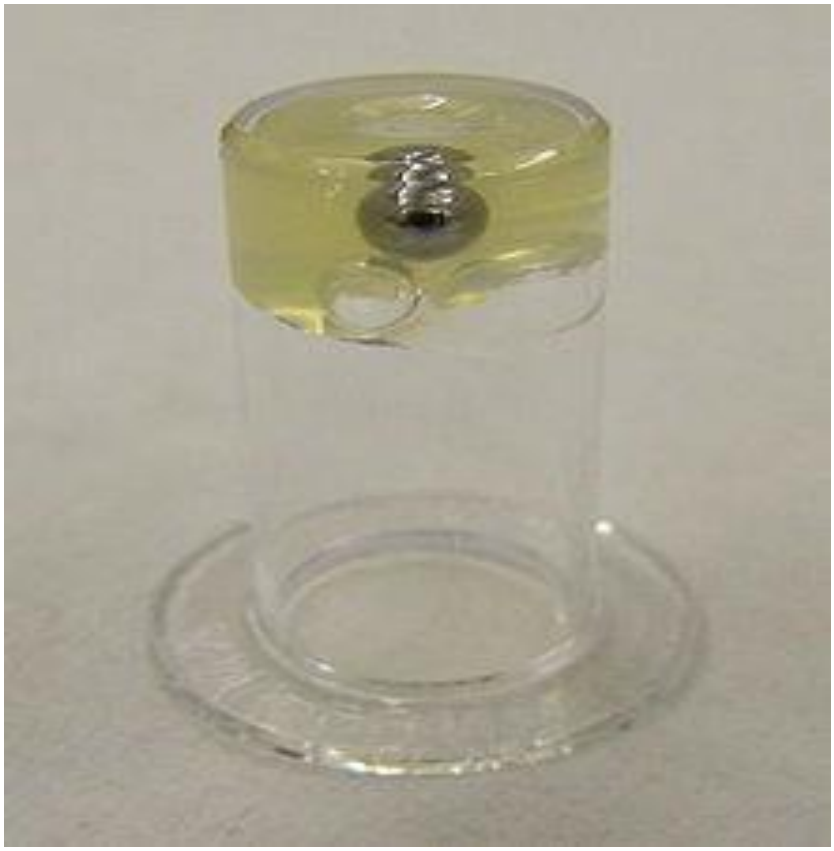
- Normal: 6 to 10 minutes
- Increased in deficiency of clotting factors

c)Prothrombin time and INR:

- Prothrombin time indicates the prothrombin in the blood
- Normal : 12 seconds
- INR is devised as a way to standardize measurements of Prothrombin time. International sensitivity index (ISI) is a assigned to each batch of tissue

factors, which indicates the activity of tissue factors with a standardized sample.

- **INR=(PT patient/PT normal)^{ISI}**
- INR normal= 0.9 to 1.3
- High INR of 4 or 5 indicates bleeding
- Low INR of 0.5 indicates clot
- Patients on warfarin, INR should be maintained between 2 and 3.



Blood plasma after the addition of tissue factors

forms a gel like structure- test for prothrombin time.

CRYOPRECIPITATE:

- It is obtained by thawing the fresh frozen plasma and centrifuging it and the precipitate obtained is cryoprecipitate.
- It is rich in fibrinogen and hence it is primarily used as a source of fibrinogen.

Composition:

Each unit of cryoprecipitate contains

a) Factor VIII : 80-120U/ concentrate

b) Fibrinogen : 150-250 mg/concentrate

c) Factor XIII : 20-30% of original FFP

d) vWF : 40-70% of original FFP

Uses:

a) Hemophilia A: since it contains factor VIII, it can be used in hemophilia A, currently Desmopressin acetate (DDAVP) or factor VIII or both is used for mild to moderate disease.

b) It can be used in von Willebrand's disorder.

c) Since it is rich in fibrinogen, it can be used for treating hypofibrinogenemia.

d) Cryoprecipitate has been used as sealant along with thrombin due to abundance of fibrinogen.

e) It is used in disseminated intravascular coagulation.

f) It can aid massive hemorrahage.

FRESH FROZEN PLASMA:

Fresh frozen plasma is obtained when citrated plasma is separated from whole blood and freezed within 8 hours of collection or citrated apheretic plasma is freezed within 6 hours.

Storage:

- when temperature is maintained at -18^0c or below, it can stored and used over a year.
- There will be minimal loss of label coagulation factors like factor V and factor VIII under these circumstances.
- Once fresh frozen plasma is thawed, it can be stored in the refrigerator for 24 hours before it can be used.

FFP contains:

a) All the coagulation factors

b) Plasma proteins

c) Glucose -535mg/dl, Na^+-12mEq/L, Cl^- 73mEq/L, K^+-3.5mEq/L, HCO_3–15mEq/l

- It is hyperosmolal, hyperglycemic, hypernatremic and hypochloremic.
- FFP contains the stable as well as labile components of the coagulation, complement and fibrinolytic systems. Moreover it contain proteins that maintain the oncotic pressure, that modulate the immunity and other proteins that have diverse activities. In addition, fats, carbohydrates and minerals are present in similar concentrations to those present in circulation.

Dose: 10-15ml/kg

Plasma frozen within 24 hours (FP24):

- The plasma which is frozen within 24 hrs has replaced the fresh frozen plasma largely.
- FP24 contents are similar to those of fresh frozen plasma, except for factor VIII which is almost about 80% less in FP24 when compared to the FFP.

Uses:

1. It is used in emergency situations where a patient is bleeding or in patients who are deficient in multiple coagulation factors undergoing an invasive procedure.
2. It can be used in single factor deficiency where specific factor is not available.
3. For reversal of warfarin therapy.
4. It is used in thrombotic thrombocytopenic purpura (TTP).
5. Disseminated intravascular coagulation and HELLP syndrome.
6. Liver diseases and vitamin K deficiencies.
7. Massive transfusion.

Demerits:

a) There is high chance of viral infectivity during transfusion including HIV infection.

b) Chance of allergic or anaphylactic reactions.

c) FFP should be ABO compatable, Rh typing can be disregarded to prevent agglutination reactions.

d) FFP are not concentrates, so when transfused in large quantities can result in volume overload and cardiac failure.

SUTURES:

Definition:

- **Surgical suture** is a medical device used to hold body tissues together after an injury or surgery. OR
- Suture is a piece of thread-like materials that is used to stitch or approximate tissues or hold the wound together until healing takes place. OR
- Any strand of material used to ligate bleeders or used to approximate tissues.

It is applied generally by using a needle with an attached length of thread. It comes in a number of different shapes, sizes and thread materials that have been developed over in millennia.

Classification of Suture Material:

- **Absorbable Suture**: An absorbable suture is one that can be digested by body cells and fluids.
- Absorption rate depends on various factors like the type of body tissue, nutritional status of the patient and the presence of infection.
- Depending on the diameter and length, absorbable suture is available in prepackaged and presterilized forms in various sizes. Sizes range from number 5, which is the heaviest to number 12-0, which is the finest. The length ranges from 12 to 60 inches.

a) Plain gut-suture material produced from the sheep gut that has not been treated to lengthen its absorption time in the tissues.

b) Chromic gut-plain catgut when treated with chromic oxide, so that it will delay its absorption or digestion.

c) Synthetic absorbable sutures.

1) Polyglactin (vicryl)

2) Polyglycolic acid (dexon)

3) Polydioxanone (PDS)

2. **Nonabsorbable Suture**: The suture material which is not absorbed during the healing process. It becomes encapsulated with tissue and remains in the body until it is removed or cast off.

- Non absorbable sutures Eg: Silk, nylon, cotton, linen, polypropylene and
- corrosion-resisting steel wire.

STAPLES:

Surgical staples are specialized devices that are used in surgery in place of sutures to close skin wounds, anastomose or to remove parts of the bowels or lung.

Stapling is much faster than suturing that is done by hand, and also more accurate and consistent.

The technique was pioneered by a Hungarian surgeon, **Humor Hultl**, who is considered as the "father of surgical stapling". **Hultl**'s prototype stapler was founded in 1908, which weighed eight pounds (3.6 kg) and was primarily devised to achieve leak proof anastomoses.

Staples are classified depending on their material or shape. Most commonly medical staples are made from titanium or stainless steel, but they can also be made from other materials like iron, chromium, nickel or plastic.

Medical staples may be straight, curved or circular.

TISSUE ADHESIVES:

A) Fibrin glue, also called **fibrin sealant**, is a formulation which is made up of fibrinogen (lyophilised pooled human concentrate) and thrombin (bovine, which is reconstituted with calcium chloride) that is used to create fibrin clot, which can be applied to the tissue sites to glue them together. Thrombin is an enzyme which takes part in the coagulation cascade and converts fibrinogen into fibrin monomers between 10 and 60 seconds giving rise to a three dimensional gel.

Factors that influence fibrin gel dimensions, giving rise to fine or coarse gel are:

1. Changing concentration of fibrinogen

2. Changing concentration of thrombin: increase in concentration increases ultimate tensile strength
3. Changing concentration of calcium
4. pH
5. Temperature

- It may also contain aprotinin, fibronectin and plasminogen.

Uses of fibrin glue:

a) Repairing dural tear.

b) Bronchial fistulas.

c) To attain hemostasis after spleen and liver trauma.

d) It can also be employed in "no sutures" corneal transplantation.

B) Cyanoacrylate:

- It is the generic name given to the family of strong fast-acting adhesives. Cyanoacrylates include methyl 2-cyanoacrylate, ethyl-2-cyanoacrylate, n-butyl cyanoacrylate and 2-octyl cyanoacrylate
- It is commonly called as "Super Glue" and "Krazy Glue".
- It is mainly used as tissue adhesive and as an alternative for approximating two cut edges of skin.

Materials and Methods

METHODOLOGY:

30 patients admitted in general surgery department and plastic surgery department in Coimbatore medical college undergoing split skin grafting will be studied prospectively between 19.11.2012 to 18.11.2013.

INCLUSION CRITERIA:

- Age of the patients- 18 years to 80 years
- Diabetic ulcers
- Hypertensive ulcers
- Traumatic ulcers
- Burns
- Post surgical raw area or Iatrogenic

EXCLUSION CRITERIA:

- Pregnancy
- Children < 18 years and adults > 80 years
- Malignant ulcers/ malignancies
- Irradiation
- Peripheral vascular diseases

- Co-morbid factors like anemia, hypertension, diabetes mellitus, etc. will be corrected where possible.
- Antibiotics to be started, as part of pre-operative treatment in all patients undergoing split skin grafting.
- Per-operatively each patient is used as the case and control, where fibrin glue is applied on one half and sutures and /or staples are applied on the other half of the same ulcer.
- Examinations of the graft for anchorage is considered and graded satisfactory or not.
- Examination of the wound will be started from **third post-operative day** onwards and will include inspection for the graft uptake, soakage and infection.
- 14^{th} day scores by both the two observers for all the parameters will be considered final.
- Examination will be continued for a minimum of 2 weeks and the scoring will be done by assessing the following three parameter, namely

    (1) Graft take

    (2) Soakage

    (3) Infection

Anchorage :

a) Satisfactory

b) Not satisfactory

Scale I: Graft take

Graft take	Scores
91-100%	**4**
81-90%	**3**
71-80%	**2**
61-70%	**1**
<60% or patchy	**0**

Scale II: Soakage

Soakage	Scores
No soakage	**3**
Partially soaked	**2**
Totally soaked	**1**

Scale III: Infection

Infection	Scores
Mild	3
Moderate	2
Frank pus	1

Post op follow up:

	Observer I(scores)				Observer II(scores)			
Parameters	3rd day	7th day	14th day	3rd month	3rd day	7th day	14th day	3rd month
Graft uptake								
Soakage								
Infection								

Results :

- Observers will give scores for both fibrin and sutures and/ staples separately.
- Average of the 14th day score given by both the observers for each parameter will be taken into account.

	Fibrin		Sutures and staples	
Parameters	**Observer I**	**observer II**	**Observer I**	**Observer II**
Graft uptake				
Soakage				
Infection				

MATERIALS AND METHODS :

The wound beds of all the 30 patients included in the study were prepared well before surgery. Once the granulation tissue is adequate and pus culture and sensitivity report showed, no growth or micro-organism load is less than10^5 /gram of tissue is achieved, the cases were posted for surgery.

Materials required:

a) Fibrin glue

b) Sutures and/staples

a)Fibrin glue: contains two component → Fibrinogen

→ Thrombin

Fibrinogen:

- cryoprecipitate was used, since it is a rich source of fibrinogen.
- There are enough literatures and articles to show that cryoprecipitate is rich source of fibrinogen.

Thrombin:

- It was prepared according to the method described by Armand J Quick.
- This was obtained from fresh frozen plasma (FFP) of healthy donors screened negative for HIV and Hepatitis B.
- 100 ml of FFP was thawed to 2- 4 ^{0}C and was 10 times diluted with distilled water, making 1000 ml of solution.
- 10 ml of 1% acetic acid was added to this to bring the pH 5.3 and this resulted in the formation of precipitate.
- It was kept for half an hour and then centrifuged at 3000 rpm for 5 minutes.
- The precipitate was collected and in this, normal saline was added to make it 100 ml and then pH was brought up to approximately 7 by titrating with sodium carbonate.
- This was put in a water bath (37 ^{0}C) and 1 ml of 0.1 M CaCl2 (Calcium Chloride) was added.
- The clot which formed in 45-120 seconds was removed by wrapping it around a stirring glass rod.
- The thrombin solution thus prepared was water clear and was constant in potency.
- The strength of thrombin was standardized to 10 second of thrombin time with the help of full strength thrombin solution. This thrombin solution was

stored in deep freeze at less than -20^{0}C to maintain the potency and could be used up to a month.

- Once the components are prepared, they were stored in deep freezer and used when required.
- Cold chain was maintained. On the day of surgery, the components were taken out from the deep freezer and thawed to room temperature. Before surgery the wound bed is prepared well. Adequate thickness of graft is harvested. One component is sprinkled over the bed and the other component on the graft and graft is applied.
- Once the graft is applied, it is checked for the anchorage by pulling it gently with forceps or moving the graft with hands and noted whether anchorage is satisfactory or not.
- Grafted limb is immobilized and paraffin gauze is applied to the donor site. Subsequently in the post-operative period, Graft is monitored for graft uptake, soakage and infection by the observers and results are tabulated.

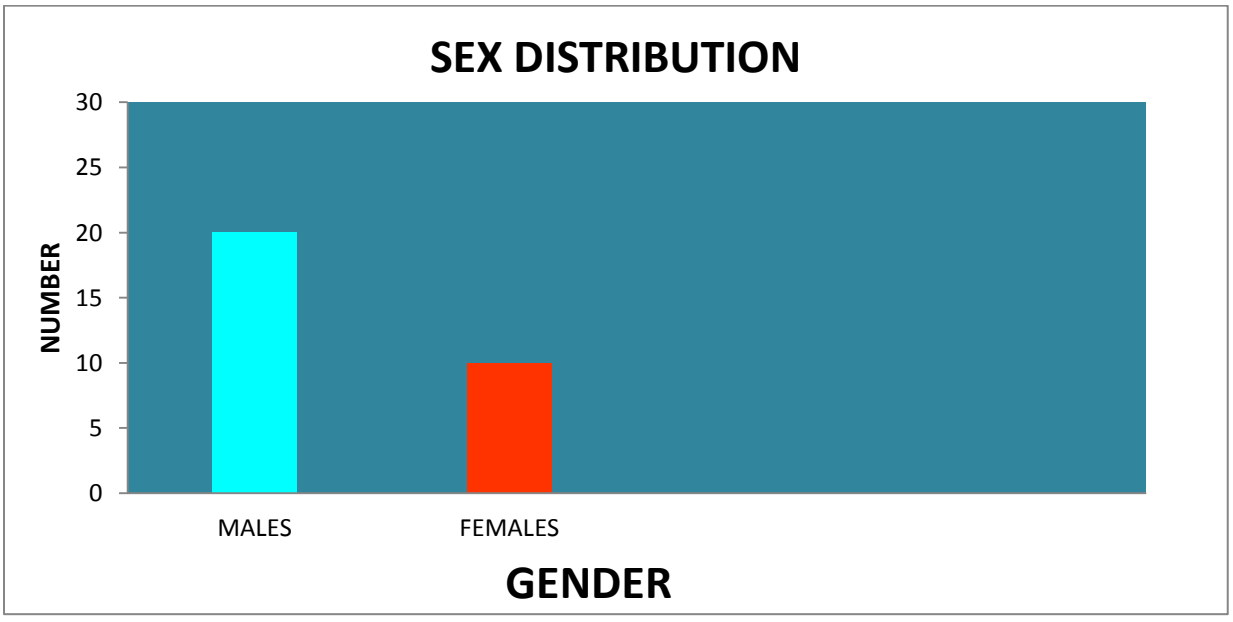
Results

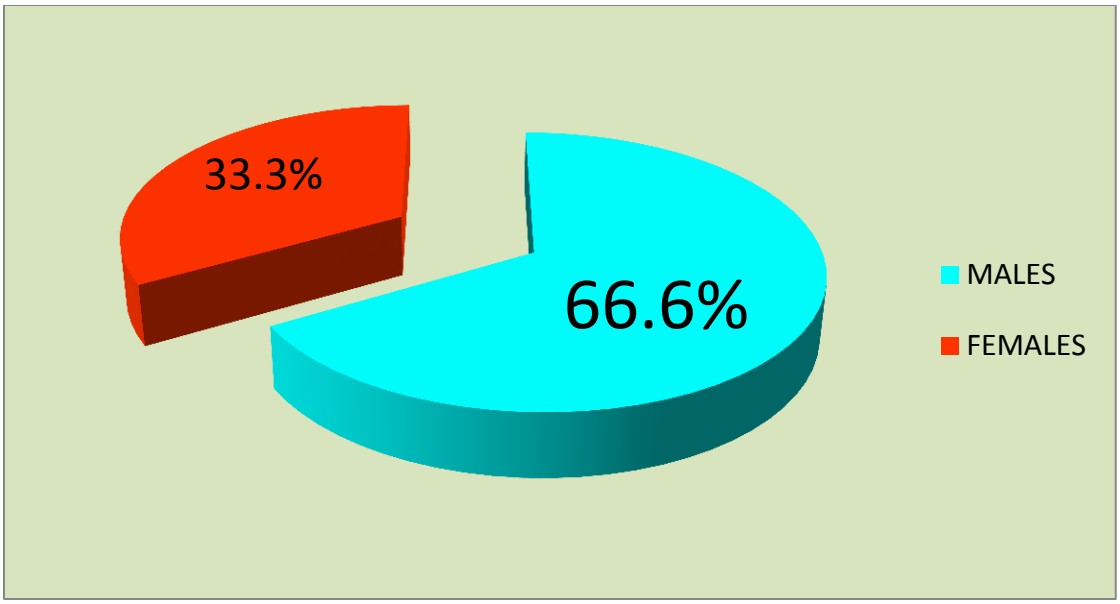
RESULTS

A) SEX DISTRIBUTION:

Total number of patients: 30

SEX	NUMBER	PERCENTAGE
Males	20	66.66%
Females	10	33.33%





Pie chart showing sex distribution

B) AGE DISTRIBUTION:

Patients aged between 31 and 80 years are included in the study

AGE IN YRS	MALE	FEMALE
31-40	1	2
41-50	5	4
51-60	8	2
61-70	5	1
71-80	1	1

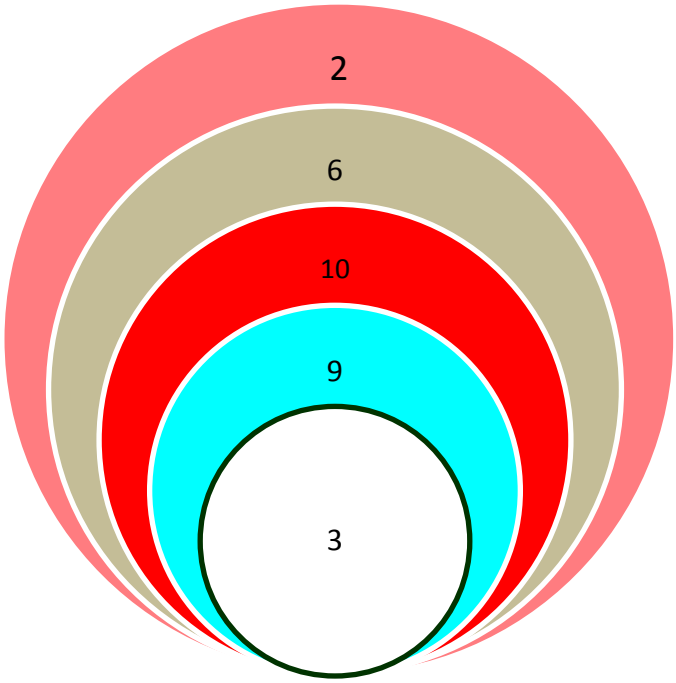


Diagram showing total number of patients in different age group

= 31- 40 YEARS

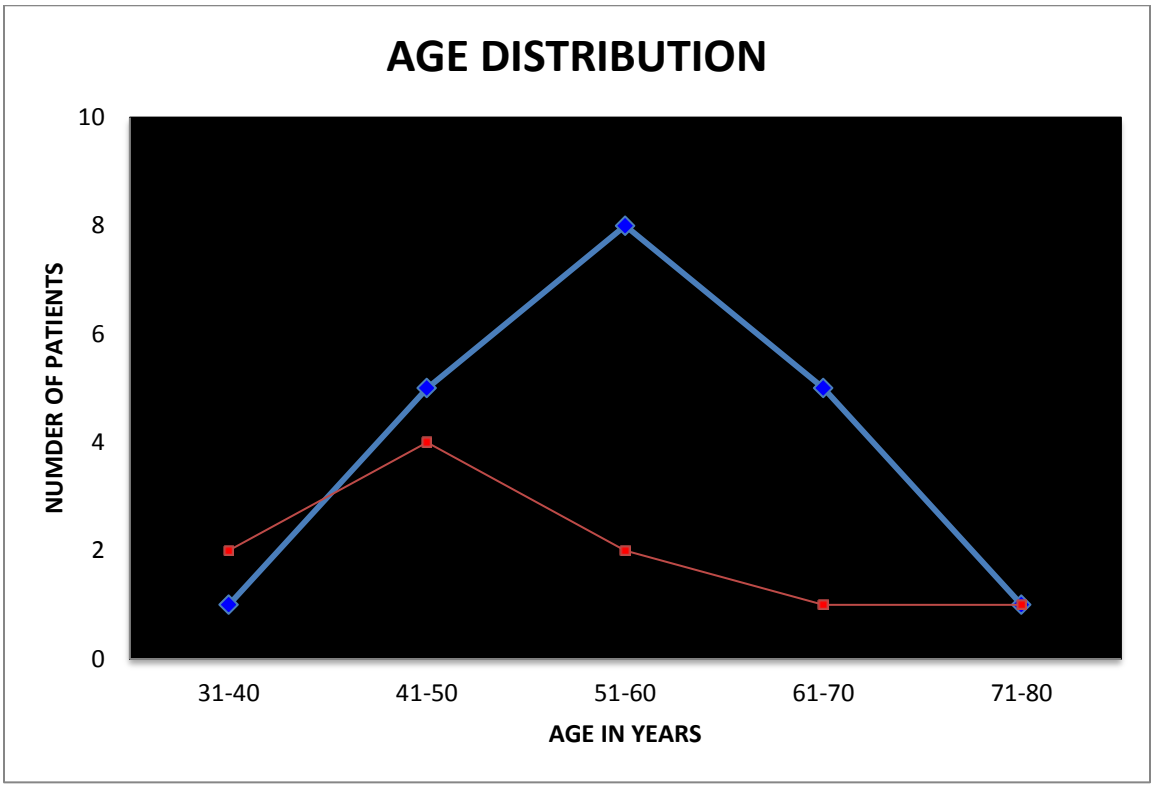
= 61-70 YEARS

= 41-50 YEARS

= 71-80 YEARS

= 51-60 YEARS

- Maximum number of patients in the study comes under 51 to 60 years.
- Least number of patients are of 71to 80 years.

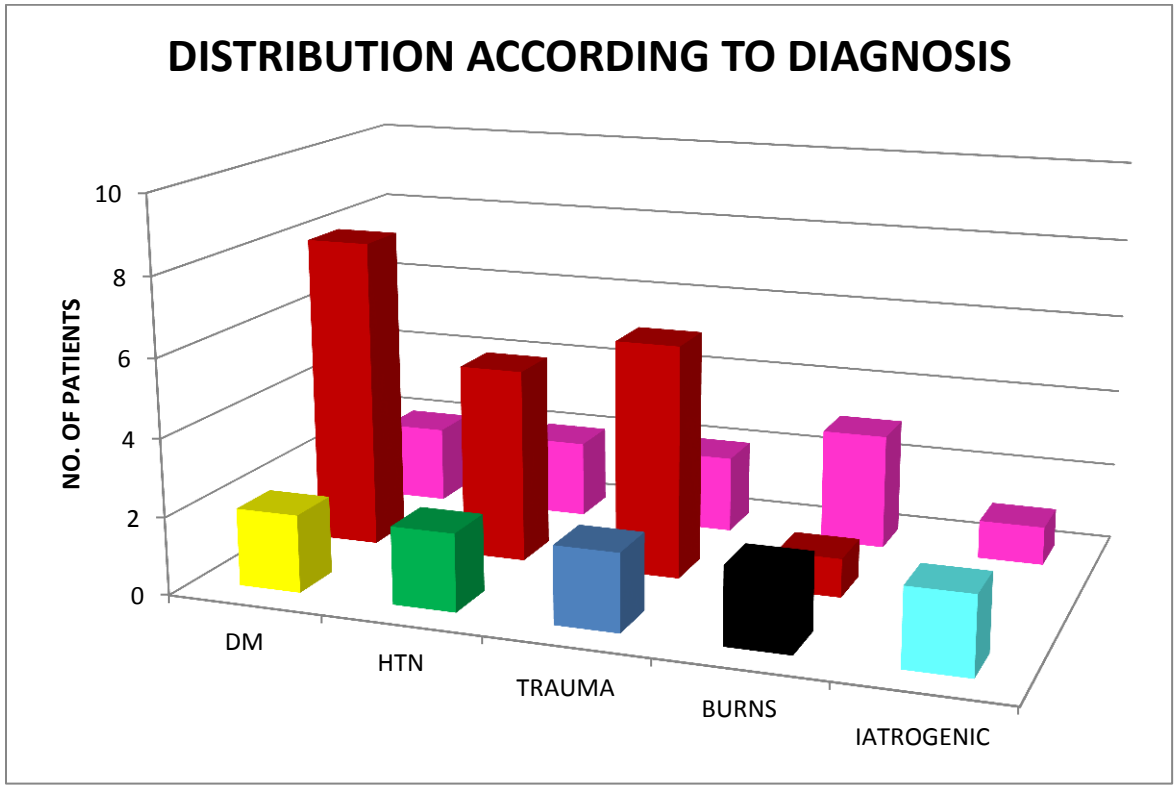


Line diagram showing male and female distribution among different age group

MALES

FEMALES

C) DISTRIBUTION ACCORDING TO DIAGNOSIS:



Bar diagram showing distribution of male and female sex according to the diagnosis

DIABETES MELLITUS

HYPERTENSION

TRAUMA

BURNS

IATROGENIC (POST SURGERY RAW AREA)

MALES

FEMALES

D) ANCHORAGE PERCENTAGE:

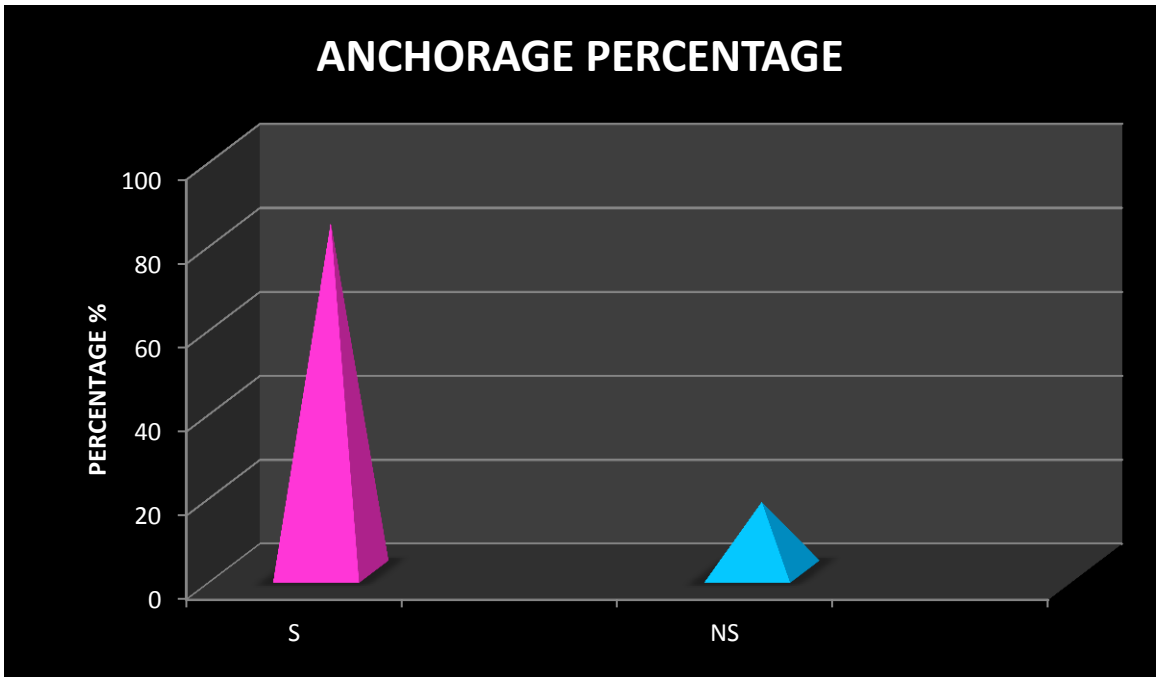


Diagram showing anchorage

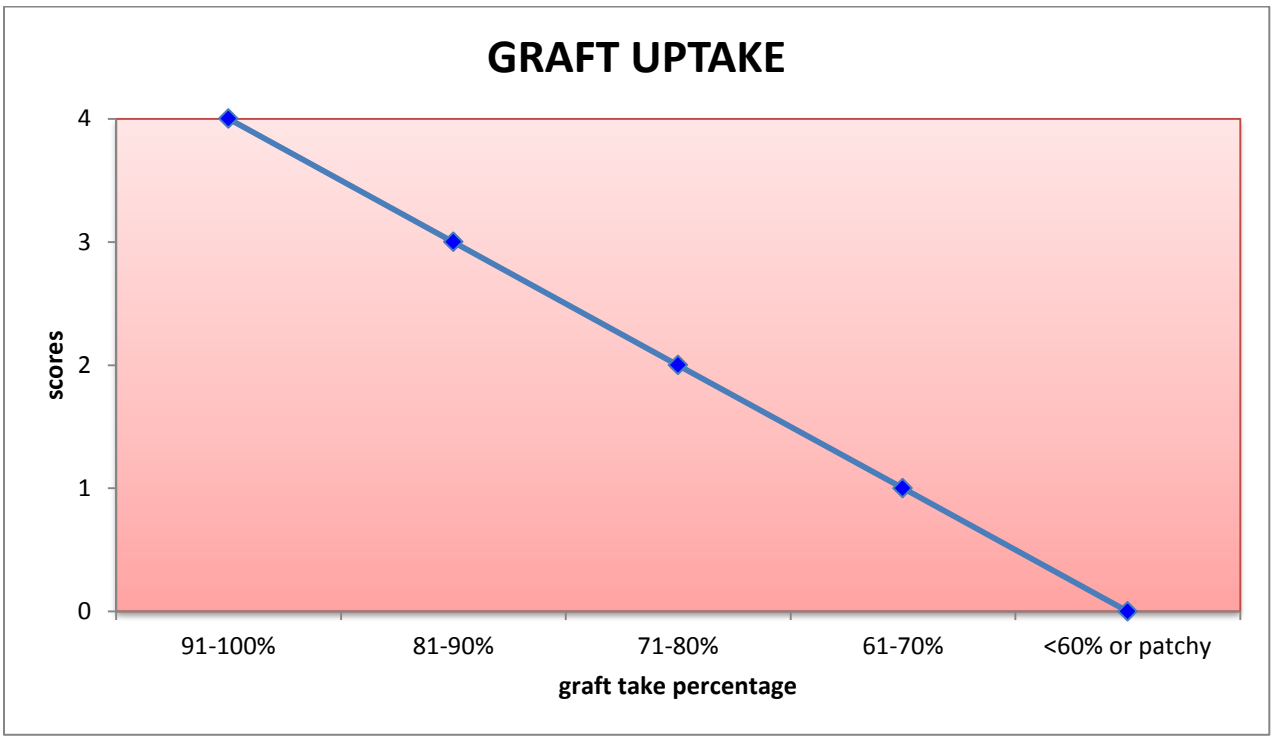
SATISFACTORY ANCHORAGE

UNSATISFACTORY ANCHORAGE

- **Anchorage is satisfactory in 83.33% of cases**

E) GRAFT UPTAKE SCORING

GRAFT TAKE	SCORE
91-100%	4
81-90%	3
71-80%	2
61-70%	1
<60% or patchy	0



Line diagram showing graft uptake scores

F) SCORING FOR SOAKAGE

SOAKAGE	SCORE
No soakage	**3**
Partially soaked	**2**
Totally soaked	**1**

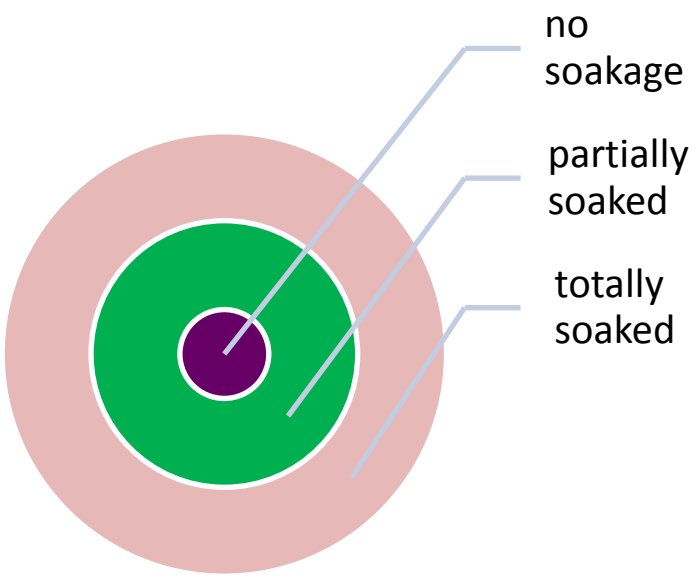


Diagram showing soakage scores

= 3

= 2

= 1

G) INFECTION SCORING:

INFECTION	SCORE
Mild	**3**
Moderate	**2**
Frank pus	**1**

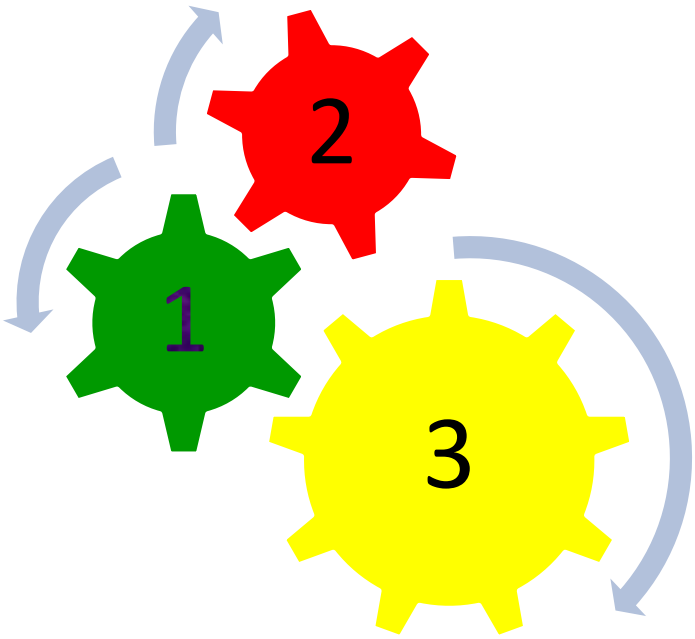


Diagram showing scores given to infection

MILD

MODERATE

FRANK PUS

H) COMPARISON BETWEEN FIBRIN AND SUTURES AMONG DIABETES MELLITUS PATIENTS:

- Total of average scores given by both the observers is taken for each parameter.
- Since the scores of fibrin is on the higher side it is taken as 100% (percentile) and compared with the sutures and staples.

Parameter	Fibrin(total scores)	Sutures N staples (total scores)
Graft uptake	35	34.5
Soakage	26	22.5
Infection	27.5	23

Parameter	Fibrin	Sutures N staples	Results of fibrin group
Graft uptake	100%	90%	10% better uptake
Soakage	100%	86.53%	13.46% less soakage
Infection	100%	83.63%	16.36% less infection

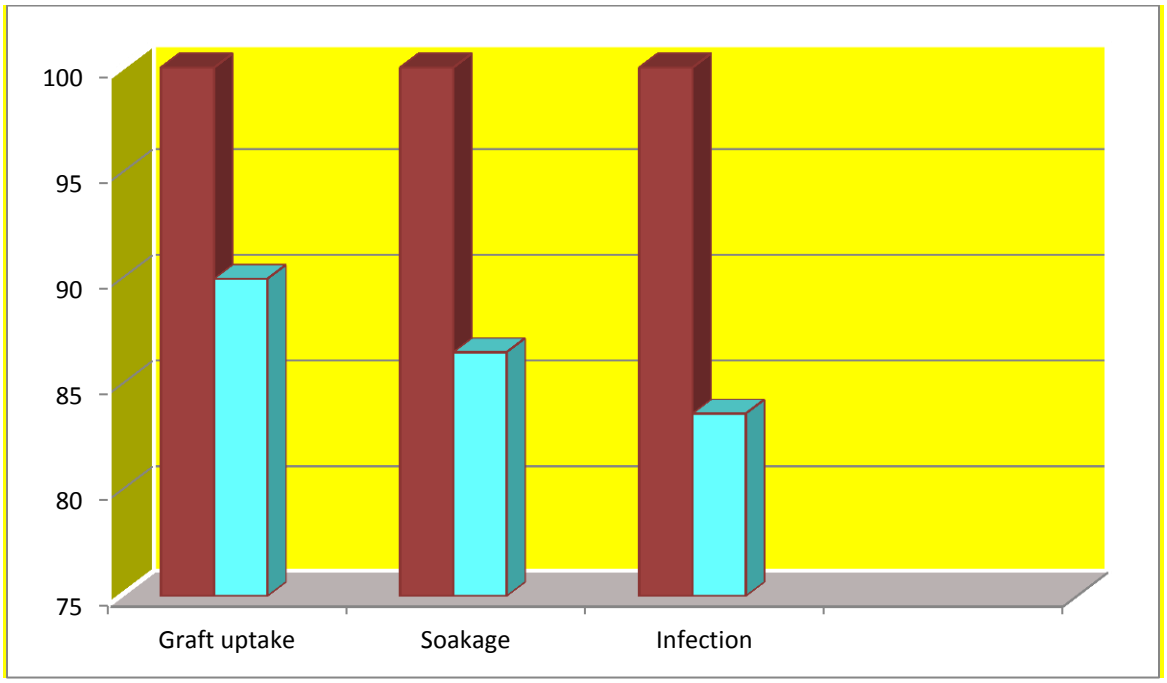


Diagram showing graft uptake, soakage and infection in fibrin and control group among diabetic patients:

- Anchorage in all diabetic patients was satisfactory.

I) COMPARISON BETWEEN FIBRIN AND SUTURES AMONG HYPERTENSIVE PATIENTS:

- Total of average scores given by both the observers is taken for each parameter.
- Since the scores of fibrin is on the higher side it is taken as 100%(percentile) and compared with the sutures and staples.

Parameter	Fibrin(total scores)	Sutures N staples(total scores)
Graft uptake	26.5	25
Soakage	19.5	18
Infection	19.5	18

Parameter	Fibrin	Sutures N staples	Results of fibrin group
Graft uptake	100%	94.33%	5.67% better uptake
Soakage	100%	92.30%	7.7% less soakage
Infection	100%	92.30%	7.7% less infection

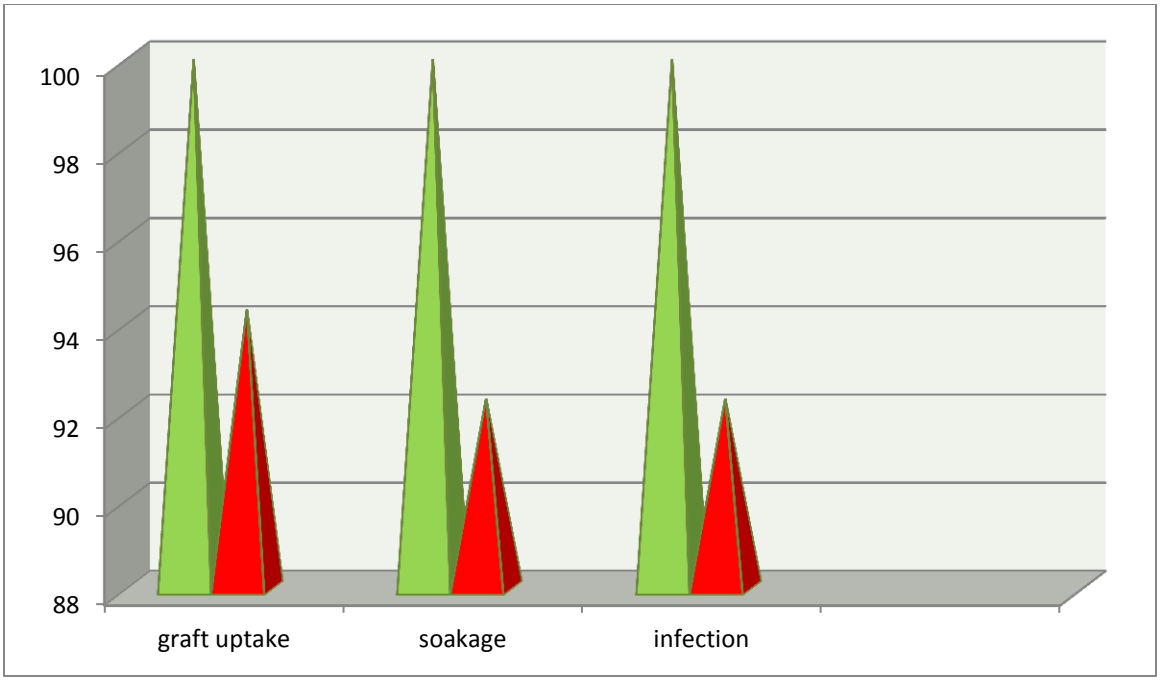


Diagram showing graft uptake, soakage and infection in fibrin and control group among hypertensive patients

- Out of 7 patients anchorage was satisfactory in 6 patients.

J) COMPARISON BETWEEN FIBRIN AND SUTURES AMONG TRAUMA PATIENTS:

- Total of average scores given by both the observers is taken for each parameter.
- Since scores of fibrin is on the higher side it is taken as 100%(percentile) and compared with the sutures and staples.

Parameter	Fibrin(total scores)	Sutures N staples(total scores)
Graft uptake	30.5	29.5
Soakage	21.5	21
Infection	23	21.5

Parameter	Fibrin	Sutures N staples	Results of fibrin group
Graft uptake	100%	96.72%	3.28% better uptake
Soakage	100%	97.67%	2.33% less soakage
Infection	100%	93.47%	6.53% less infection

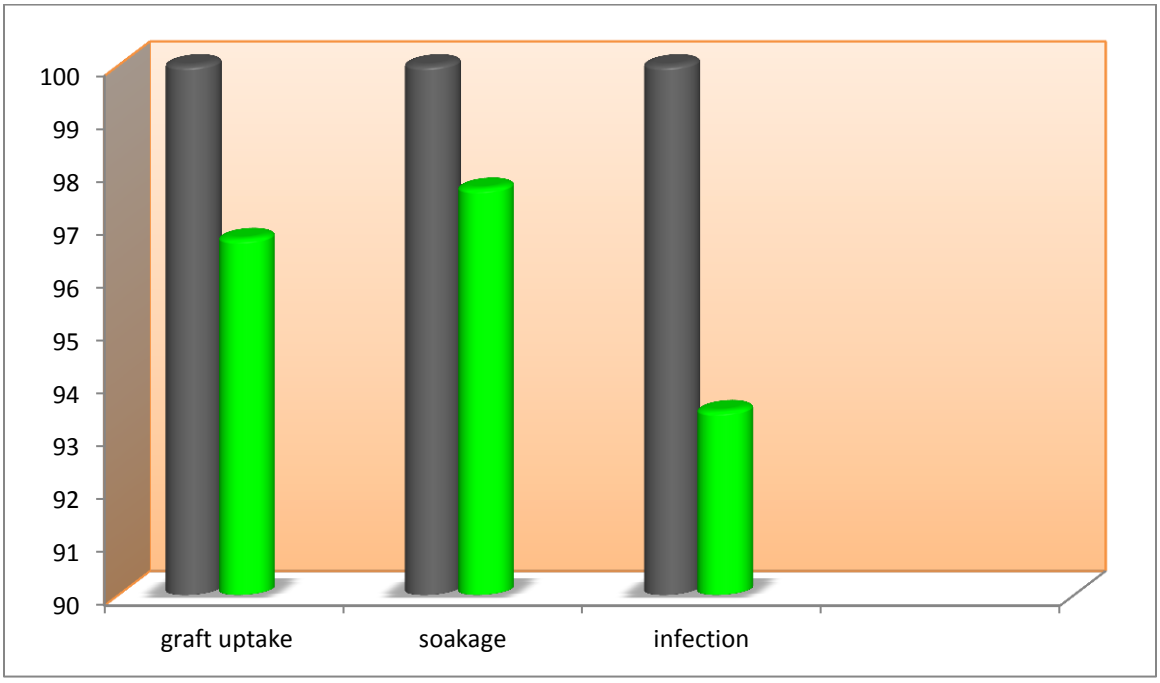


Diagram showing graft uptake, soakage and infection in fibrin and control group among trauma patients:

- Anchorage was satisfactory in all patients.

K) COMPARISON BETWEEN FIBRIN AND SUTURES AMONG BURNS PATIENTS:

- Total of average scores given by both the observers is taken for each parameter.
- Since scores of fibrin is on the higher side it is taken as 100%(percentile) and compared with the sutures and staples.

Parameter	Fibrin(total scores)	Sutures N staples(total scores)
Graft uptake	11.5	11
Soakage	8	7
Infection	8	8

Parameter	Fibrin	Sutures N staples	Results of fibrin group
Graft uptake	100%	95.65%	4.35% better uptake
Soakage	100%	87.5%	12.5% less soakage
Infection	100%	100%	No difference

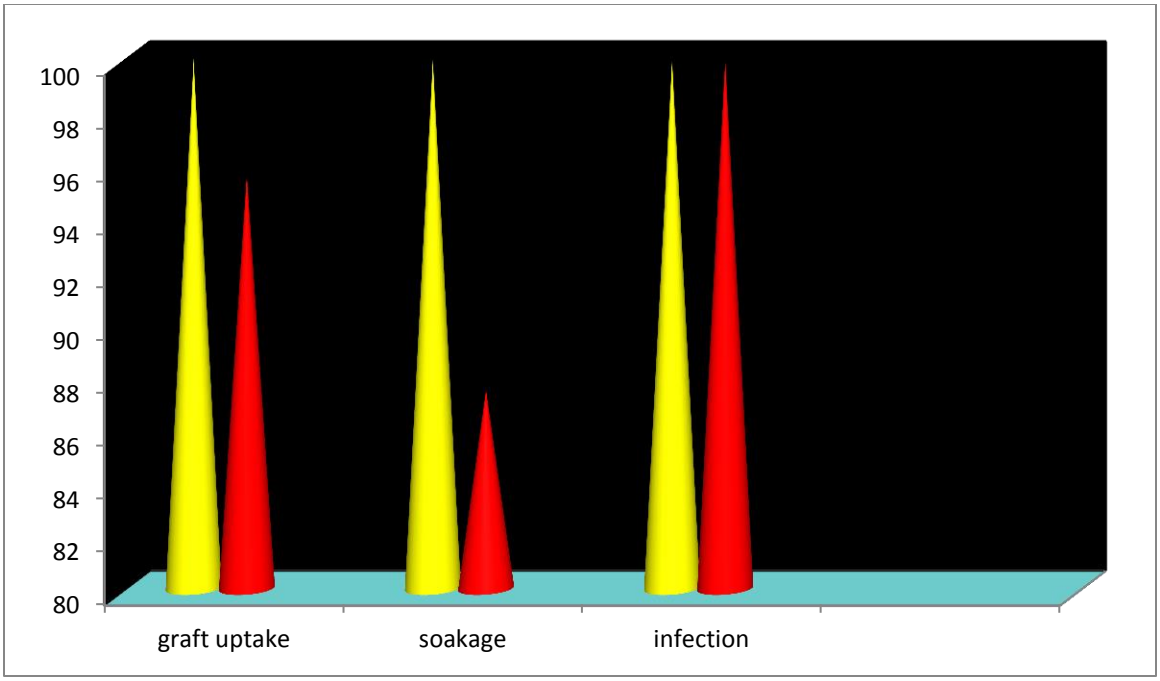


Diagram showing graft uptake, soakage and infection in fibrin and control group among burns patients

- Anchorage was not satisfactory in burns patients.

M) COMPARISON BETWEEN FIBRIN AND SUTURES AMONG POST SURGERY RAW AREA PATIENTS:

- Total of average scores given by both the observers is taken for each parameter.
- Since scores of fibrin is on the higher side it is taken as 100%(percentile) and compared with the sutures and staples.

Parameter	**Fibrin(total scores)**	**Sutures N staples(total scores)**
Graft uptake	**3.5**	**3**
Soakage	**3**	**3**
Infection	**3**	**3**

Parameter	Fibrin	Sutures N staples	Results of fibrin group
Graft uptake	100%	85.7%	14.29% better uptake
Soakage	100%	100%	No difference
Infection	100%	100%	No difference

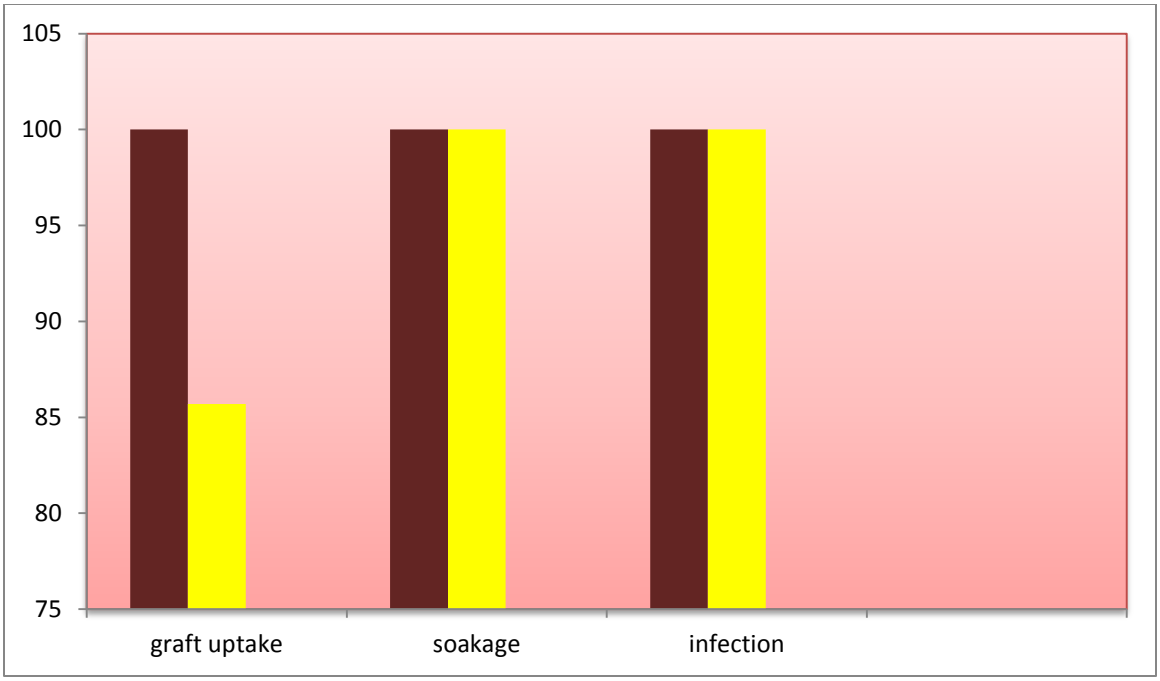


Diagram showing graft uptake, soakage and infection in fibrin and control group among post surgery raw area patient

- Anchorage was satisfactory

STATISTICS:

Since we are comparing two samples in a same individual for one parameter, we are joining the measurements or pairing it, in a sensible way to get a result. So we have chosen **PAIRED T TEST** in our study.

Paired t test formula:

$$t = \frac{\Sigma d}{\sqrt{\frac{n\left(\Sigma d^2\right)-\left(\Sigma d\right)^2}{n-1}}}$$

The top of the formula is the sum of the differences (i.e. the sum of *d*). The bottom of the formula reads as the square root of the following n times the sum of the differences squared minus the sum of the squared differences, all over

n-1.

a) The sum of the squared differences: $\sum d^2$ means take each difference in turn, square it, a add up all those squared numbers.

b) The sum of the differences squared: $(\sum d)^2$means add up all the differences and square t result.

c) Brackets around something in a formula mean (do this first), so $(\sum d)^2$means add up all t differences first, then square the result.

GRAFT UPTAKE			
	MEAN	S.D	NO.
Fibrin	3.57	0.47	30
Sutures and staples	3.33	0.51	30

PAIRED T TEST

T- test	Degree of freedom	Probability	significance
3.751	29	0.001	P<0.01

P<0.01 – Significant at 1% level.

SOAKAGE			
	MEAN	S.D	NO.
Fibrin	2.60	0.46	30
Sutures and staples	2.38	0.55	30

PAIRED T TEST

T- test	Degree of freedom	Probability	significance
3.067	29	0.005	P<0.01

P<0.01 – Significant at 1% level.

INFECTION			
	MEAN	S.D	NO.
Fibrin	2.70	0.43	30
Sutures and staples	2.45	0.48	30

PAIRED T TEST

T- test	Degree of freedom	Probability	significance
3.340	29	0.002	P<0.001

P<0.01 – Significant at 1% level.

Pre operative picture

Intra operative picture

Serous discharge No **Serous discharge**

STAPLED AREA **FIBRIN GLUE APPLIED AREA**

Discussion

DISCUSSION

- Wound healing by primary intention is the most desirable one**.** This has been the challenge for surgeons to achieve these feet since ages. To overcome this sutures were developed, but the expectations and dream of developing an ideal suture was not completely fulfilled. So we started looking for alternatives, that's when we created tissue adhesives. Today we have both biological (Fibrin glue) and synthetic (Cyanoacrylate).
- In our study we have used fibrin glue as tissue adhesive for fixing split skin grafting.
- The principle behind using fibrin glue as tissue adhesive is born from the concept that, the first phase of inflammation involves formation of thrombus through a series of events that takes place in coagulation cascade.
- In our study we have used fibrin glue, that is of human origin and bypassing the problem of anaphylactic reaction, which is encountered when fibrin glue of bovine origin is used.
- The process of using fibrin glue in our setting was easy and comfortable, only loose string is preparation of thrombin from fresh

frozen plasma. Once thrombin is prepared, it is stored under -20^{0}c to maintain the potency and can be used over a month. This helped to overcome the difficult and cumbersome method of conventional suturing and pain associated with stapling.

- Our study is based on 30 patients, which mainly comprises of

  Males-20 patients, which accounts to 66.66% and Females-10 patients, which accounts to 33.33% and this male dominance is consistent with world population.
- Most of the patients were in the age group of 51-60years which accounts for 33.3% and least among 71-80 years accounting for 6.66%. Others 31-40years=10%, 41-50years=30% and 61-70years= 20%.
- Out of the 30 patients in our study, diabetes mellitus dominated the strength of patients accounting for 33%, out of which 26.4% were males and 6.6% were females, indicating the shift of diabetic epidemic towards India.
- Strength of trauma in our study was 26.66% with males being 19.99% and females-6.66%.
- Hypertension association in our study was 23.33% with male population being 16.66% and females making up to 6.66%.

- Only 4 burns patients participated in the study, out of 75% were females and one male patient. With one post modified mastectomy raw area patient in the study.
- Anchorage was satisfactory in 83.33% of the cases, except for burns patients and one patient with hypertension, where anchorage was not satisfactory. Anchorage in our study was comparable to study done by Saxena et al [1], Varanasi, India in fixing split skin grafting/ flaps using fibrin glue. The results were consistent and satisfactory when compared to this study. Anchorage of the graft to bed was rapid, the time consumed for the procedure was very short when compared to conventional suturing and/ or stapling, these can be corroborated with those of Marcos et al [2].
- Overall graft uptake was better in fibrin glue study group. Also soakage and infection was less in fibrin glue study group, when compared to the conventional suturing /stapling.
- Among diabetic patients, graft uptake was 10% better, 13.46% less soakage and 16.36% less infectious when compared with the control group.

- In hypertensive patients, graft uptake was better by 5.67% and superiority among soakage and infection with 7.7% less soakage and 7.7% less infection.
- When post traumatic raw area group was considered, graft taken was 3.28% better with 2.33% less soakage and 6.53% less infection.
- Though the anchorage was not so satisfactory among the burns patients, graft uptake was 4.35% better, 12.5% less seroma formation when compared to control group. But no difference in infection was found among both the groups.
- In post surgical raw area patient, no difference in soakage and infection was found, but the graft uptake was significantly better by 14.29%.
- Graft uptake in our study is similar to the study conducted by the Saxena et al [1]. The findings in our study also corroborates with those of Machado et al[3], where fibrin glue was used for skin grafting and second intention wound healing following dermatalogic surgeries like excision of malignant epithelial cutaneous tumors.
- Soakage in our study among cases was very less when compared to the control group. These findings were comparable to Saxena et al [1] study. There are more evidence, when fibrin glue was used as

a sealant in Abdominoplasty, seroma and soakage formation was less according to the study conducted by the Marcos et al [2]. According to Dragan et al study [4], fibrin glue can be used as sealant to prevent pocket related complications in patients undergoing pacemaker tansplantations. This shows that fibrin glue is a very good sealant or a hemostatic agent, seroma formation following its application will be very minimal. There are enough literature and studies to show that it is very efficacious as a sealant.

- Even though diabetic patients are prone for infections, infection rate among diabetic patients were less in fibrin glue study group following grafting. This can be corroborated to those of study conducted on rats by Jabs et al [5] , where they inoculated wound with staphylococcus and grafts was placed. The graft was still better among infected wounds.

  In our study no difference in infections rate was found among burns and postsurgical raw area patients. In burns patient the bed was devascularised and in postsurgical raw area patient, the bed supplied enough nutrition for equal take of graft.

Conclusion

CONCLUSION:

- This is a Prospective comparative study.
- Thirty patients were included in the study with male preponderance.
- Patients aged between 31 and 80 years were included in the study, with highest influence of patients between 51 and 60 years accounting for 33.3% and least among 71-80 years age group accounting for 6.66%.
- Major strength of the study was covered by diabetics and trauma patients.
- Anchorage was rapid and excellent in most of the patients except for burns patients were standards were not met.
- Graft uptake, soakage and infection rate among all the patients in fibrin glue group was better and satisfactory compared to control group, expect no difference in infection rate among burns and post surgical raw area patients and no difference in soakage in post surgical raw area patient was found.
- We have advocated paired T test in our study and results were significant, proving that the application of fibrin glue in fixing skin grafting when compared to conventional method of suturing and stapling is simple, efficacious, cost-effective, less time consuming, better graft uptake, less seroma formation, minimum infection, easy and comfortable intra-operative and post-operative care and patients can be made ambulant early.

- Hence we conclude that this can be considered as a very good alternative to conventional suturing and stapling.

Appendix -I

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Appendix -II

PROFORMA

Name: DOA:

Age/ Sex: DOS:

Occupation: DOD:

Address:

CHIEF COMPLAINTS:

1. Ulcer over extremities- onset and progress
2. H/o burns
3. H/O trauma

PAST HISTORY

1. H/O DM/ HTN/ Asthma/ TB/ Epilepsy
2. H/O malignancy/irradiation.
3. H/O chronic cardiac disease/chronic liver disease/ chronic renal disease/vasculitis

PERSONAL HISTORY*:*

1. H/O of present conception
2. Smoker/ Alcoholic
3. Bowel and bladder habits, sleep pattern.

GENERAL PHYSICAL EXAMINATION*:*

1. Hydration
2. Nutritional status
3. Pallor
4. Icterus
5. Cyanosis/ clubbing/ edema
6. Generalized/ regional lymphadenopathy
7. Pulse rate
8. Blood pressure.

SYSTEMIC EXAMINATION*:*

- **Cardiovascular system:**

  Inspection

  Auscultation

- **Respiratory system***:*

  Inspection

  Percussion

  Auscultation

- **Central nervous system***:*

  Consciousness

  Orientation

  Higher mental functions

- **Per abdomen***:*

  Inspection

  Auscultation

LOCAL EXAMINATION:

Inspection*:*

- Number, site, extent, shape and size
- Margins- undermined/ punched out/ sloping/ rolled out or everted
- Floor of the ulcer
- Discharge
- Adjacent area

Palpation:

- Temperature and tenderness
- Margins- Induration and base
- Mobility
- Examination of regional lymph nodes
- Examination for vascular diseases- peripheral pulses
- Examination for nerve lesions

Others:

Ankle brachial index

Body mass index

Diagnosis:

Investigations:

1. Complete hemogram, random blood sugar, blood urea, serum creatinine and serum electrolytes.
2. Liver function tests, serum proteins
3. pus culture and sensitivity
4. Electrocardiogram

5. Chest radiograph- PA view
6. Test for HIV I & II, HBsAg
7. And relevant investigations for associated medical conditions

CONSENT FORM

It has been explained to me in my mother tongue and I completely understand my condition, its related complications and the treatment options available. I have been explained in detail regarding this study- 'TWO COMPONENT PREPARATION OF FIBRIN GLUE AND ITS CLINICAL EVALUATION IN SPLIT SKIN GRAFTING". I hereby give my voluntary and willful consent to participate in the above mentioned study.

DATE: PLACE:

SIGNATURE OF THE PATIENT (WITH NAME)

SIGNATURE OF THE RELATIVE (WITH NAME)

SIGNATURE OF THE WITNESS (WITH NAME)

MASTER CHART

SL.NO	NAME	AGE/ SEX	DIAGNOSIS	SIZE	ANCHORAGE		OBSERVATION DAY	GRAFT UPTAKE				SOAKAGE				INFECTION				RESULTS (14 TH DAY)					
					S	NS														GRAFT UPTAKE		SOAKAGE		INFECTION	
								FIBRIN		S&S		FIBRIN		S&S		FIBRIN		S&S		FB	SNS	FB	SNS	FB	SNS
								I	II	I	II	I	II	I	II	I	II	I	II						
1	PALANISAMY	64Y/M	TRAUMATIC ULCER	20X8	✓		3 rd	4	4	4	4	2	3	2	2	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3
2	THANGAMANI	48Y/F	POST MRM RAW AREA	10X 10	✓		3 rd	4	4	4	4	2	2	2	2	3	3	3	3						
							7 th	4	3	3	3	3	3	3	3	3	3	3	3						
							14 th	4	3	3	3	3	3	3	3	3	3	3	3	3.5	3	3	3	3	3
3	SELVARAJ	54Y/M	DIABETIC FOOT	12X6	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	3	3	3	3	2	2	3	3	2	2						
							14 th	4	4	3	3	3	3	2	2	3	3	2	2	4	3	3	2	3	2
4	RANGASAMY	42Y/M	HYPERTENSIVE ULCER	6X4		✓	3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3
5	MARIMUTHU	52Y/M	TRAUMATIC ULCER	12X8	✓		3 rd	4	4	4	4	2	2	2	2	3	3	3	3						
							7 th	3	4	3	3	2	2	2	2	3	3	2	2						
							14 th	3	4	3	3	2	2	2	2	3	3	2	2	3.5	3	2	2	3	2

S= satisfactory; NS= not satisfactory; S and S= sutures and staples; FB= fibrin

SL. NO	NAME	AGE/ SEX	DIAGNOSIS	SIZE	ANCHORAGE		OBSERVATION DAY	GRAFT UPTAKE				SOAKAGE				INFECTION				RESULTS (14 TH DAY)					
					S	NS														GRAFT UPTAKE		SOAKAGE		INFECTION	
								FIBRIN		S&S		FIBRIN		S&S		FIBRIN		S&S							
								I	II	I	II	I	II	I	II	I	II	I	II	FB	SNS	FB	SNS	FB	SNS
6	MURUGESAN	46Y/M	DIABETIC ULCER	10X 10	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	3	3	3	3	2	2	3	3	2	2						
							14 th	4	4	3	3	3	3	2	2	3	3	2	2	4	3	3	2	3	2
7	VASANTHI	51Y/F	TRAUMATIC ULCER	10X 8	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3
8	KARUPPASAMY	44Y/M	TRAUMATIC ULCER	20X6	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	3	4	3	2	2	2	2	3	2	3	2						
							14 th	4	3	4	3	2	2	2	2	3	2	3	2	3.5	3.5	2	2	2.5	2.5
9	BALAMURUGAN	41Y/M	BURNS	40X 30		✓	3 rd	3	3	3	3	2	2	2	2	3	3	3	3						
							7 th	2	2	2	2	2	2	1	1	2	2	2	2						
							14 th	2	2	2	2	2	2	1	1	2	2	2	2	2	2	2	1	2	2
10	DANIEL	59Y/M	HYPERTENSIVE ULCER	10X6	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	3	3	3	3	2	2	3	3	2	2						
							14 th	4	4	3	3	3	3	2	2	3	3	2	2	4	3	3	2	3	2

S= satisfactory; NS= not satisfactory; S and S= sutures and staples; FB= fibrin

SL. NO	NAME	AGE/ SEX	DIAGNOSIS	SIZE	ANCHORAGE		OBSERVATION DAY	GRAFT UPTAKE				SOAKAGE				INFECTION				RESULTS (14 TH DAY)					
					S	NS														GRAFT UPTAKE		SOAKAGE		INFECTION	
								FIBRIN		S&S		FIBRIN		S&S		FIBRIN		S&S							
								I	II	I	II	I	II	I	II	I	II	I	II	FB	SNS	FB	SNS	FB	SNS
11	BALLAN	61Y/M	DIABETIC ULCER	14X8	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	3	3	3	3	2	2	2	2	2	2	2	2						
							14 th	3	3	3	3	2	2	2	2	2	2	2	2	3	3	2	2	2	2
12	KUPPATHAL	63Y/F	DIABETIC ULCER	10X 8	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	3	3	3	3	2	2	2	3	3	2	2						
							14 th	4	3	3	3	3	2	2	2	3	3	2	2	3.5	3	2.5	2	3	2
13	KANNAN	70Y/M	DIABETIC ULCER	8X6	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	3	4	3	3	2	2	2	2	3	3	2	2						
							14 th	3	4	3	3	2	2	2	2	3	3	2	2	3.5	3	2	2	3	2
14	ABU BAKKAR	51Y/M	DIABETIC ULCER	14X8	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	3	4	3	2	2	2	2	3	3	3	3						
							14 th	4	3	4	3	3	3	3	3	3	3	3	3	3.5	3.5	3	3	3	3
15	SIVAKUMAR	57Y/M	HYPERTENSIVE ULCER	10X6	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3

S= satisfactory; NS= not satisfactory; S and S= sutures and staples; FB= fibrin

SL. NO	NAME	AGE/ SEX	DIAGNOSIS	SIZE	ANCHORAGE		OBSERVATION DAY	GRAFT UPTAKE				SOAKAGE				INFECTION				RESULTS (14 TH DAY)					
					S	NS														GRAFT UPTAKE		SOAKAGE		INFECTION	
								FIBRIN		S&S		FIBRIN		S&S		FIBRIN		S&S							
								I	II	I	II	I	II	I	II	I	II	I	II	FB	SNS	FB	SNS	FB	SNS
16	KAMATCHI CHETTIYAR	78Y/M	DIABETIC ULCER	12X4	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	3	3	3	3	3	2	2	3	3	3	2						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	3.5	3	3	2	3	2.5
17	ANWAR	32Y/M	TRAUMATIC ULCER	20X 12	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3
18	RAJENDRA	48Y/M	DIABETIC ULCER	10X8	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	3	4	3	4	3	2	3	2	3	2	3	2						
							14 th	3	4	3	4	3	2	3	2	3	2	3	2	3.5	3.5	2.5	2.5	2.5	2.5
19	BANNARI	42Y/F	BURNS	20X 15		✓	3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	3	3	3	3	2	2	2	2	2	2	2	2						
							14 th	3	3	3	3	2	2	2	2	2	2	2	2	3	3	2	2	2	2
20	VELUSAMY	60Y/M	HYPERTENSIVE ULCER	8X6	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3

S= satisfactory; NS= not satisfactory; S and S= sutures and staples; FB= fibrin.

SL. NO	NAME	AGE/ SEX	DIAGNOSIS	SIZE	ANCHORAGE		OBSERVATION DAY	GRAFT UPTAKE				SOAKAGE				INFECTION				RESULTS (14 TH DAY)					
					S	NS														GRAFT UPTAKE		SOAKAGE		INFECTION	
								FIBRIN		S&S		FIBRIN		S&S		FIBRIN		S&S							
								I	II	I	II	I	II	I	II	I	II	I	II	FB	SNS	FB	SNS	FB	SNS
21	TAMILSELVAN	54Y/M	TRAUMATIC ULCER	22X 14	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	3	3	3	2	3	2	2	2	3	2	2						
							14 th	4	3	3	3	2	3	2	2	2	3	2	2	3.5	3	2.5	2	2.5	2
22	ANITHA	33Y/F	BURNS	20X 18		✓	3 rd	3	3	3	3	3	3	3	3	3	3	3	3						
							7 th	3	3	3	3	2	2	2	2	2	2	2	2						
							14 th	3	3	3	3	2	2	2	2	2	2	2	2	3	3	2	2	2	2
23	PERIASAMY	66Y/M	TRAUMATIC ULCER	15X7	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3
24	SUMITHA	48Y/F	BURNS	24X 16		✓	3 rd	4	3	4	3	2	2	2	2	3	2	2	2						
							7 th	4	3	3	3	2	2	2	2	2	2	2	2						
							14 th	4	3	3	3	2	2	2	2	2	2	2	2	3.5	3	2	2	2	2
25	NATCHI MUTHU	67Y/M	DIABETIC ULCER	11X5	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	3	4	4	3	3	3	3	3	3	3	3	3						
							14 th	3	4	4	3	3	3	3	3	3	3	3	3	3.5	3.5	3	3	3	3

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SL. NO	NAME	AGE/ SEX	DIAGNOSIS	SIZE	ANCHORAGE		OBSERVATION DAY	GRAFT UPTAKE				SOAKAGE				INFECTION				RESULTS (14 TH DAY)					
					S	NS														GRAFT UPTAKE		SOAKAGE		INFECTION	
								FIBRIN		S&S		FIBRIN		S&S		FIBRIN		S&S							
								I	II	I	II	I	II	I	II	I	II	I	II	FB	SNS	FB	SNS	FB	SNS
26	LAKSMI	72Y/F	HYPERTENSIVE ULCER	10X5	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	3	3	3	3	2	2	2	2	2	2	2	2						
							14 th	3	3	3	3	2	2	2	2	2	2	2	2	3	3	2	2	2	2
27	RATHINAM	51Y/M	HYPERTENSIVE ULCER	9X 4	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3
28	KALPANA	38Y/F	DIABETIC ULCER	13X9	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	3	3	3	3	2	2	2	2	2	2	2	2						
							14 th	3	3	3	3	2	2	2	2	2	2	2	2	3	3	2	2	2	2
29	CHRISTINA	51Y/F	HYPERTENSIVE ULCER	8X4	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	3	3	3	3	2	2	2	3	2	2	2						
							14 th	4	3	3	3	3	2	2	2	3	2	2	2	3.5	3	2.5	2	2.5	2
30	NIRMALAMMA	41Y/F	TRAUMATIC ULCER	8X6	✓		3 rd	4	4	4	4	3	3	3	3	3	3	3	3						
							7 th	4	4	4	4	3	3	3	3	3	3	3	3						
							14 th	4	4	4	4	3	3	3	3	3	3	3	3	4	4	3	3	3	3

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